ENHANCED DISSOLUTION AND IN-VIVO BIAVAILABILITY OF ITRACONAZOLE β – CYCLODEXTRIN COMPLEX

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of degree of

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Submitted by
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CHAPTER -I

INTRODUCTION

1. INTRODUCTION

Oral route is the most preferred for administration of drugs owing to its known advantage of better patient compliance. Drugs administered by oral route should dissolve in gastric and intestinal fluid before they permeate through the membrane of GI tract and enter into systemic circulation. So solubility is a prerequisite for absorption of drugs.

Poorly soluble drugs poses dissolution rate limited absorption. In other words in-vitro dissolution is the index of in-vivo absorption of poorly soluble drugs. Enhancement of solubility of poorly soluble drugs improves dissolution and absorption with improved bioavailability and better therapeutic action and patient compliance. A number of methodologies adopted to improve solubilization of poorly water-soluble drugs further to improve its bioavailability. The techniques generally employed for solubilization of drugs include micronization, chemical modification, pH adjustment, solid dispersion complex, co-solvency, micellar solubilization, hydrotropy etc.

Itraconazole is an orally active triazole antifungal agent[1]. It demonstrates broad spectrum of antifungal activity against a number of fungal species including dermatophytes. *Malasesia fur-fur*, *Candida species, Aspergillus species, and histoplasma, capsulatum var, capsulatum*.[2] It acts by selective distruption of cytochrome P-450 mediated ergosterol synthesis in fungal membrane and there by leads to cell death.[3] Itraconazole is an extremely weak base (pKa = 3.7) which is virtually unionized at physiological pH.[4,5] The drug is poorly soluble and so its efficacy is severely limited. Absorption is enhanced by concurrent administration of food.[6] similarly ketoconazole absorption is markedly inhibited by agents which increases gastric pH.[7] Improvement of the oral bioavailability of Itraconazole has been problematic. However concentration of Itraconazole in serum may be variable and suboptimal, there by potentially compromising therapy. Indeed treatment failures have been associated with low concentration of Itraconazole in blood [8] because of the hydrophobic structure of all azoles. This may adversely affect concentration in blood when given orally. Therefore, enhanced dissolution of itraconazole will be beneficial to improve its absorption and bioavailability following oral administration.

Complexation is one of the approaches followed to improve solubility of poorly soluble drugs. β -Cyclodextrin a product of enzymatic degradation of starch by *Bacillusmacerans,* has been used in pharmaceutical field to improve the solubility of hydrophobic compounds. β -Cyclodextrin interact with hydrophobic molecules and form a non- covalent inclusion complex that lowers the chemical potency of the molecule in solution and thus enhances the solubility of the molecule. Pharmaceutical modification of drug molecule by inclusion complex with cyclodextrins has been extensively developed to improve solubility, dissolution rate, chemical stability, absorption and bioavailability of poorly water soluble drugs and reduces side effects and toxicity of drugs.[9-17]

βs-cyclodextrins has been used to form inclusion complex with itraconazole.[18] Pyroxicam and miconazole, [19, 20] ibuprofen [21] and showed improved dissolution of those drugs. In a previous study[22] an improved dissolution at pH 1.4 and bioavailability of Itraconazole β-cyclodextrin inclusion complex prepared by supercritical carbon did-oxide method has been reported in animal model. It has also been documented that absorption of Itraconazole is enhanced by concurrent administration of food.[6] the gastric pH increases in the presence of food and influence the absorption and bioavailability of Itraconazole. Upon reviewing the literature, the influence of varied pH conditions in acidic environment of the stomach on the dissolution and bioavailability of Itraconazole β-cyclodextrin complex is lacking and therefore the present study was attempted to investigate the same. Itraconazole β-cyclodextrin inclusion complex was prepared by co precipitation method in different concentrations of β-cyclodextrins and evaluated for in-vitro dissolution and in-vivo bioavailability in comparison with physical mixture and pure drug. The study may offer scope for understanding the bioavailability behaviour of Itraconazole in varied pH conditions of the stomach and its therapeutic efficacy.

CHAPTER - II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The therapeutic effectiveness of a drug depends up on the bioavailability of drug molecule. Solubility is one of the parameter to achieve desired therapeutic concentration of drug. **The term 'solubility' is defined as maximum amount of solute that can be dissolved in a given amount of solvent.** In the Biopharmaceutical Classification System (BCS - II) drugs with poor aqueous solubility are poorly bioavailable. It has been estimated that 40% of the new chemical entities currently being discovered are of poor water-solubility.[23]

Many poorly soluble drugs are either basic or acidic in character and thus exhibit structural components which can be protonated or deprotonated, respectively. In the GIT, the local pH environment varies from the upper small intestine to the distal colon, the drug absorption depending upon whether the molecule is predominantly acidic or basic. For example, in a predominantly acidic environment, a weak acidic will remain unionised and thus be absorbed via the passive trans-cellular route. The pH of the buffer media can strongly influence the solubility of such compounds, and thus the required permeability values are achieved. To develop dosage form, oral route of drug delivery is the simple and most convenient way of administration of all the drugs, because of more patient compliance, less side effects and more stable. There are many techniques to improve the solubility of poorly soluble drugs, such as solubilisation**,** chemical modification, size reduction**,** formation of inclusion complexes. [24]

Noyes-Whitney equation:

Illustrates how the dissolution rate of even very poorly soluble compounds might be improved to minimize the limitations to oral bioavailability:

$$dc/dt = AD(Cs- c)/h$$

Where

dC/ dt is the rate of dissolution,

A is the surface area available for dissolution,

D is the diffusion coefficient of the compound,

Cs is the solubility of the compound in the dissolution medium,

C is the concentration of drug in the medium at time t .

H is the thickness of the diffusion layer.

s**Techniques for solubility enhancement**

There are various techniques available to improve the solubility of hydrophobic drugs. Some traditional and novel approaches to improve the solubility are:

1. Particle Size Reduction
2. Solid Dispersion
3. Nanosuspension
4. Supercritical Fluid Technology
5. Cryogenic Technology
6. Inclusion Complex Formation Techniques
7. Floating Granules

β-Cyclodextrins:

Cyclodextrins are cyclic oligosaccharides containing at least 6 D-(+) glucopyranose units attached by α-(1, 4) Glucosidic bonds. (CDs), They were first discovered in 1891,[25] various approaches that have been used to improve the solubility and dissolution rate of drugs, complexation with cyclodextrins is one of the most promising ones, which enhance their solubility, dissolution rate, chemical stability and bioavailability and reduce their side effects and toxicity. Preparation of solid inclusion complexes between cyclodextrins and various drugs includes kneading, co-evaporation, sealed-heating, co-grinding, spray-drying and freeze-drying.[26] the use of supercritical carbon dioxide (sc co_2) has been recently proposed for the preparation of various drug-cyclodextrin inclusion complexes for enhanced solubility and dissolution rate.

Cyclodextrins are able to form solid inclusion complexes (host–guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation.[25] In these complexes, a guest molecule is held within the cavity of the cyclodextrin host molecule. Complex formation is a dimensional fit between host cavity and guest molecule.[27] The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes.[28] No covalent bonds are broken or formed during formation of the inclusion complex.[29] The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state.[30]

S.No	Category	Example of carriers
1.	Polymers	Polyvinylpyrrolidone, Polyvinylpolypyrrolidone, Polyvinyl alcohol, Polyethylene glycols, Hydroxypropyl methylcellulose, Hydroxypropyl cellulose, Poly (2-hydroxyethylmethacrylate), Methacrylic copolymers (Eudragit® S100 sodium salts and Eudragit® L100 sodium salts).
2.	Superdisintegrants	Sodium starch glycolate, Croscarmellose sodium, Cross-linked polyvinylpyrrolidone, Cross-linked alginic acid, Gellan gum, Xanthan gum, Calcium silicate.
3.	Cyclodextrins	β -Cyclodextrins, Hydroxypropyl-β-cyclodextrins.
4.	Carbohydrates	Lactose, Soluble starch, Sorbitol, Mannitol, β-(1-4)-2-amino-2-deoxy-D-glucose (Chitosan), Maltose, Galactose, Xylitol, Galactomannan, British gum, Amylodextrin.
5.	Surfactants	Poloxamers (Lutrol® F 127, Lutrol® F 68), Polyglycolized glyceride (Labrasol), Polyoxyethylene sorbitan monoesters (Tweens), Sorbitan esters (Spans), Polyoxyethylene stearates, Poly (beta-benzyl-L-aspartate) -b- poly (ethylene oxide), Poly (caprolactone) -b- poly (ethylene oxide).
6.	Hydrotropes	Urea, Nicotinamide, Sodium benzoate, Sodium salicylate, Sodium acetate, Sodium-o-hydroxy benzoate, Sodium-p-hydroxy benzoate, Sodium citrate.
7.	Polyglycolized glycerides	Gelucire 44/14, Gelucire 50/13, Gelucire 62/05
8.	Acids	Citric acid, Succinic acid, Phosphoric acid.
9.	Dendrimers	Starburst® polyamidoamine (PAMAM).

Table 1: Different types of polymers/ carriers

Types of inclusion complexes

1. Physical blending method
2. Kneading method
3. Co-precipitation technique
4. Solution/solvent evaporation method
5. Neutralization precipitation method
6. Milling/Co-grinding technique
7. Atomization spray drying method
8. Lyophilization/ spray drying method
9. Microwave irradiation method
10. Supercritical anti- solvent technique

1. Physical blending method:

A solid physical mixture of drug and CDs are prepared simply by mechanical trituration. In laboratory scale CDs and drug are mixed together thoroughly by trituration in a mortar and passes through appropriate sieve to get the desired particle size in the final product. In industry scale, the preparation of physical mixtures is based on extensive blending of the drug with CDs in a rapid mass granulator usually for 30 minutes. These powdered physical mixtures are then stored in the room at controlled temperatures and humidity conditions. [31]

2. Kneading method:

This method is based on impregnating the CDs with little amount of water or hydro alcoholic solutions to converted into a paste. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through sieve if required[32]. (Parik et al.)[33] have reported the dissolution enhancement of nimesulide using complexation method. In laboratory scale kneading can be achieved by

using a mortar and Pestel.[34-36] In large scale the kneading can be done by utilizing the extruders and other machines. This is the most common and simple method used to prepare the inclusion complexes and it presents very low cost of production.

3. Co-precipitation technique:

This method involves the co-precipitation of drug and CDs in a complex. In this method, required amount of drug is added to the solution of CDs. The system is kept under magnetic agitation with controlled process parameters and the content is protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of the structure water from the inclusion complex. Moyano et.al.[37] studied the solid-state characterization and dissolution characteristics of gliclazide-bete-cyclodextrin inclusion complexes.

This technique leaves a drug-CD solution in very close conditions to the saturation and through abrupt changes of temperature with addition of organic solvents. It is obtained to the precipitation of the material forming inclusion complex. The powders are obtained by rotation or filtration with heat while stirring the solution.[38] However, due to low yield, risk of using organic solvents, and longer time required for the preparation in larger scale, this method is attaining little attraction in the industrial scale.[39]

4. Solution/solvent evaporation method:

This method involves dissolving of the drug and CDs separately in to two mutually miscible solvents, mixing of both solutions to get molecular dispersion of drug and complexing agents and finally evaporating the solvent under vacuum to obtain solid powdered inclusion compound. Generally, the aqueous solution of CDs is simply added to the alcoholic solution of drugs. The resulting mixture is stirred for 24 hours and evaporated under vaccum at 45 ºc. The dried mass was pulverized and passed through a 60-mess sieve. This method is quite simple and economic both on laboratory and large scale production and is considered alternative to the spray drying technique.[31]

5. Supercritical anti-solvent technique:

In the supercritical fluid anti-solvent technique, carbon dioxide is used as anti-solvent for the solute but as a solvent with respect to the organic solvent. The use of supercritical carbon dioxide is advantageous as its low critical temperature and pressure makes it attractive for processing heat-labile pharmaceuticals. It is also non-toxic, non-flammable, in-expensive and is much easier to remove from the polymeric materials. Supercritical particle generation processes are new and efficient route for improving bioavailability of pharmaceutically active compounds.[40] in addition, supercritical fluid processes were recently proposed as a new alternative method for the preparation of drug cyclodextrin complexes.

In this technique, first, drug and CD are dissolved in a good solvent then the solution is fed into a pressure vessel under supercritical conditions, through a nozzle (i.e. sprayed into supercritical fluid anti-solvent). When the solution is sprayed in to supercritical fluid anti-solvent, the anti-solvent rapidly diffuses into that liquid solvent as the carrier liquid solvent counter diffuses into the anti-solvent. Because of the supercritical fluid expanded solvent has lower solvent power than the pure solvent, the mixture becomes supersaturated resulting in the precipitation of the solute and the solvent is carried away with the supercritical fluid flow.[41,42]

Cyclodextrins as permeation enhancers:

CDs can also be used as membrane permeability enhancer and stabilizing agents [43, 44]. The permeability of the poorly soluble drugs through biological membrane is enhanced by the presence of cyclodextrins. Masson[45] these acts as permeation enhancers by carrying the drug through the aqueous barrier which exists before the lipophilic surface of biological membranes.[46] this can also be achieved through the double characteristics of the CDs, thus present character much lipophilic as hydrophilic. CDs can also be used as nasal permeation enhancers acting by interaction with nasal epithelium by modifying tight junction & lipid and protein content of the membrane, which enhances the permeation of the membrane.[47] CDs, can also be utilized as permeation enhancer in

pulmonary drug delivery systems. Rifampicin is a so- called concentration-dependent antibiotic, the rate and extent of bacterial kill is related to the attainment of high maximum concentration relative to the minimal inhibitory concentration. The rifampicin-CD inclusion compound can improve the lung transport of drug when nebulized with compatible pulmonary deposition and achieve required concentration of drug in bronchi-alveolar epithelium lining-fluid when administered as aerosolized [48-51]

Advantages of β-cyclodextrins:

CDs have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs and to increase their bioavailability and stability. In addition, CDs have been used to reduce or prevent gastrointestinal or ocular irritation, reduces unpleasant smells or tastes, prevent drug - drug or drug-additive interactions, or even to convert oils and liquid drugs into microcrystalline or amorphous powders.

1. **Enhancement of solubility:** CDs increase the aqueous solubility of many poorly soluble drugs by forming inclusion complexes with their polar molecules or functional groups. And observe water-soluble CD drug complex.[52]

2. **Enhancement of bioavailability:** Poorly bioavailable dugs are complexed with CD, dissolution rate and, absorption is enhanced. Reducing the hydrophobicity of drugs by CD complexation also improves their per-cutaneous or rectal absorption.[53]

3. **Improvement of stability:** CD complexes are improving the chemical, physical and thermal stability of drugs.[54]

4. **Reduction of irritation:** Drug substances that irritate the stomach, skin or eye can be encapsulated within a CD cavity to reduce their irritancy. Inclusion complexation with CDs reduces the local concentration of the free drug. So reduce the irritation.

5. **Prevention of incompatibility:** Drugs are often incompatible with each other or with other inactive ingredients present in a formulation. Encapsulating one of the incompatible ingredients within a CD molecule prevent drug-drug or drug-additive interaction.[55]

6. **Odour and taste masking:** Unpleasant odour and bitter taste of drugs can be masked by complexation with CDs.

7. **Material handling benefits:** Substances that are oils/liquids at room temperature can be difficult to handle and formulate into stable solid dosage forms. By Complexation with CDs may convert such substances into microcrystalline or amorphous powders which can be conveniently.[56]

Table 2: Analytic methods for characterization of solid forms

S.no	Methods	Significance
1.	Thermal analysis a. Cooling Curve Method b. Thaw Melt Method c. Thermo microscopic Method d. Zone Melting Method e. DSC Studies f. DTA Studies	To study the morphology and degree of crystallinity. To find out the interaction between drug and carrier and formation of inclusion complex.
2	X-ray Powder Diffraction Studies	To find out the crystalline or amorphous form of drug.
3	FTIR, NMR, Raman spectra	To find out the complex formation between drug and carrier.
4	Scanning Electron Microscopy	To find out the particle size and shape.
5	Dissolution rate / diffusion rate studies	Rate and extent of dissolution.
6	Thermodynamic study	Degree of crystallinity

Pharmacology of fungal infection.[57]

Fungal infections are termed as mycoses. Fungal infections are generally classified in to two types

1. Superficial fungal infections.
2. Systemic fungal infections.

1. Superficial fungal infections:

These superficial fungal infections are classified in to two types

a. Dermato mycoses.
b. Candidiasis.

Dermatomycoses are infections of the skin, hair and nails. Most commonly caused by Trichophyton, microsporum and epidermophyton spp. which caused by various types of "ring worm" or tine a, tine a capitis affect the scalp. Tinea cruris, groin, Tinea pedis. In superficial candidiasis the yeast like organism infects the mucous membrane of the mouth, vagina, and skin.

2. Systemic fungal infections

Systemic fungal infections are caused by yeast like organism. Other more serious conditions are cryptococcal meningitis or endocarditis invasive pulmonary aspergillosis is now a leading cause of death.

Drugs used in fungal infection

1. Superficial acting drugs:

Terbinafine, Clotrimazole, Nystatin, Miconazole nitrate.

2. Systemically acting drugs:

Fluconazole, Itraconazole, Amphotericine –B, Flucytosine

Griseofulvin, Potassium iodide, Terbinafine.

REVIEW OF ITRACONAZOLE

1. Improved solubility of Itraconazole in water significantly increased as the concentrations of HP-β-CYD increases, showing an AP type phase solubility diagram. The upward curvature closely corresponded to the simulation curve which was calculated on the basis of the 1:2 (guest: host) complexation model. The 1:2 complexes were formed even in the presence of 10% v/v propylene glycol, although the co-solvent system made the interaction with HP-β-CYD weaker due to the competitive inclusion. The ultraviolet spectroscopic studies also supported the 1:2 complex formation of Itraconazole with HP-β-CYD in 10% v/v propylene glycol: water solution at pH 2.0 the 1H-NMR spectroscopic studies suggested that the triazole and triazolone moieties of Itraconazole are involved in the 1:2 inclusion complexation. **K. Uekama.,** ***et al;*** **(1999)**[58]

2. Solid dispersion of Itraconazole was prepared by various pH-independent and pH-dependent hydrophilic polymers are characterized by differential scanning calorimetry, powder X-ray diffraction and scanning electron microscopy. Of the polymers tested, pH-dependent hydrophilic polymers, AEA® and Eudragit® E 100, resulted in highest increases in drug solubility (range, 141.4–146.9-fold increases). The shape of the solid dispersion particles was spherical, with their internal diameter ranging from 1–10 mm. The dissolution rate of Itraconazole from the tablets prepared by spray drying (SD-T) was fast, with \90% released within 5 min. SD-T prepared with AEA® or Eudragit® E 100 at a 1:1 drug to hydrophilic polymer ratio (w:w) showed approximately 70-fold increases in the dissolution rate over a marketed product. © 1999 Published by Elsevier Science B.V. **Sun Dong Yoo.,** ***et al;(*****1999).**[59]

3. Solid solutions of Itraconazole, a water insoluble antifungal, for improved dissolution and improved bioavailability. Influence of processing factors on drug and carrier properties in solid solution and subsequently on drug dissolution

behavior was also studied. An optimized solid solution formulation was compared with marketed product in healthy human subjects under fasted and fed conditions for bioequivalence. Polyethylene glycol (PEG) and drug were made into a solid solution at 120 °C. The cooled, solid solution was then ground into granules of different sizes. Solid solutions of lower drug concentration dissolved at a faster rate, and drug dissolution improved considerably with increasing molecular weight of PEG. Initial treatment of Itraconazole with the wetting agent/co-solvent glycerol prior to making Itraconazole into solid solution improved drug dissolution, and also reduced the PEG amount required to dissolve drug to form solid solution. Addition of a polymer such as HPMC to the solid solution eliminated precipitation of drug following dissolution. As the granule size of the solid solution was reduced, precipitation of drug during dissolution became prominent. Equivalence of two formulations could not be shown for pharmacokinetic parameters *C*max and AUC, under both fasting and fed conditions. **Kapsi., *et al;* (2001)** [60]

4. Solid dispersions containing different ratios of Itraconazole and Hydroxypropyl methylcellulose (HPMC) were prepared by solvent casting. Based on dose, differential scanning calorimetric and dissolution results, a drug/polymer ratio of 40/60 w/w was selected in order to prepare dispersions by melt extrusion. The melt extrusion process was characterized using a design of experiments (DOE) approach. All parameter settings resulted in the formation of an amorphous solid dispersion whereby HPMC 2910 5 Map's prevents re-crystallization of the drug during cooling. Dissolution measurements demonstrated that a significantly increased dissolution rate was obtained with the amorphous solid dispersion compared to the physical mixture. The outcome of DOE further indicated that melt extrusion is very robust with regard to the Itraconazole/HPMC melt extrudate characteristics. Stability studies demonstrated that the Itraconazole/HPMC 40/60 w/w milled melt extrudate formulation is chemically and physically stable for periods in excess of 6 months as indicated by the absence of degradation products or re-crystallization of the drug. **Geert verreck., *et al;* (2003)** [61]

5. Solid dispersion formulations of Itraconazole in human volunteers in comparison with Sporanox®, the marketed form. Solid dispersions made up of Itraconazole (40%, w/w) and HPMC 2910, Eudragit E100 or a mixture of Eudragit E100-PVPVA64 were manufactured by hot-stage extrusion and filled in gelatin capsules. The formulations were tested in eight human volunteers in a double blind, single dose, and cross-over study. Concentrations of the drug and its metabolite hydroxy Itraconazole in the plasma were determined using HPLC. The in vivo performance was evaluated by comparing the mean area under the plasma concentration–time curves (AUC), the mean maximum plasma concentration (*C*max), and the mean time to reach *C*max (*T*max). The mean bioavailability of Itraconazole was comparable after administration of the HPMC solid dispersion, compared to Sporanox®, while it was lower after administration of the Eudragit E100 or Eudragit E100-PVPVA64 dispersions. Due to high variability, a significant decrease in AUC and *C*max was only observed for the Eudragit E100-PVPVA formulation. Although the solid dispersions showed different in vitro dissolution behavior, *T*max values were comparable. The same observations with respect to AUC, *C*max and *T*max could be made for hydroxy Itraconazole. The present results indicate that hot-stage extrusion can be considered as a valuable alternative for manufacturing solid dispersions of Itraconazole. **Guy Van den Mootera, *et al;* (2005)** [62]

6. Reported an improve the solubility and dissolution rate of a poorly water-soluble drug Itraconazole, by Cooling curve method was used to determine the eutectic point of drug-Poloxamer 188 mixture and the phase diagram of the binary system was constructed. Solid dispersions of Itraconazole were prepared by the hot melt method and characterized by differential scanning calorimetry (DSC). Solubility and dissolution studies in various media were conducted with pure Itraconazole, a physical mixture and solid dispersions. Solubility studies indicated that poloxamer 188 increased significantly the solubility of Itraconazole in water (range 33.40- to 176.8-fold increases). The eutectic mixture resulted in highest increases in drug dissolution rate. The cumulative release of Itraconazole from solid dispersions within 60 min was 4.66 times higher than the pure drug in 0.1 ml HCl. The dissolution of Itraconazole from solid dispersions in Poloxamer 188 with a eutectic

composition reached a satisfactory level (above 90%) after 20 min in both water (0.5% SDS) and pH 6.58 PBS buffer (0.5% SDS). An increased solubility and dissolution ate of Itraconazole can be achieved by forming a eutectic mixture using Poloxamer 188 as a carrier. The drug dissolution rate was highest at a drug-polymer ratio of 5: 95 (w/w). **Siling Wang .,** ***et al ;*** **(2006).**[63]

7. Self-emulsifying drug delivery system (SEDDS) composed of oil, surfactant and co-surfactant for oral administration of Itraconazole was formulated, and its physicochemical properties and pharmacokinetic parameters of Itraconazole were evaluated. Among the surfactants and oils studied, Transcutol\, Pluronic\ L64 and tocopherol acetate were chosen that showed the maximal solubility to Itraconazole. The solubility of Itraconazole was further improved by the addition of hydrochloric acid. Droplet size of Itraconazole emulsion was kept constant both in simulated gastric fluid without pepsin (pH 1.2) and simulated intestinal fluid (pH 6.8) throughout 120-min incubation period. Itraconazole in the SEDDS rapidly dissolved in every dissolution medium whereas the Sporanox\ showed different dissolution patterns during the 120-min incubation according to the dissolution media. In fasted and fed normal diet group, AUC0Y24 h and the mean maximum plasma level (Cmax) of Itraconazole after oral administration of SEDDS in rats were comparable to those of Itraconazole after oral dose of Sporanox\. However, in fed lipid diet group, AUC and Cmax after oral administration of SEDDS in rats were 3.7- and 2.8-fold higher, respectively, compared with those of Sporanox\. These results demonstrate that the SEDDS of Itraconazole composed of Transcutol\, Pluronic\ L64 and tocopherol acetate greatly enhanced the bioavailability of Itraconazole after the dose, particularly not influenced by food intake or not. Thus, this system may provide a useful dosage form for oral water-insoluble drug without food effect. **Chong –kook Kim.,** ***et al ;*** **2006.** [64]

8. An enhanced dissolution and bioavailability characteristics of Itraconazole. By Inclusion complexes between Itraconazole and β-cyclodextrin were prepared using simple physical mixing, conventional co precipitation method, and supercritical

carbon dioxide (SC CO2). Effects of process variables (temperature, pressure) and drug: cyclodextrin ratio on inclusion yield and thermal behavior of the solid complexes prepared by SCCO2 were studied and compared to those obtained by physical mixing and co precipitation methods. In addition, dissolution amounts of the products obtained by different methods were measured in gastric fluid. Finally, pharmacokinetic studies of the inclusion complexes were conducted in male Wister rats to assess the bioavailability of the prepared complexes. Results showed that temperature, pressure and Itraconazole: cyclodextrin ratio had significant effects on the inclusion yield of the complex prepared by SC CO2 method. Higher inclusion yields were obtained in the SC CO2 method as compared to physical mixing and co precipitation methods. *In vivo* drug pharmacokinetic studies showed that the Itraconazole-β-cyclodextrin product prepared using SC CO2 gave higher bioavailability of Itraconazole (in blood, liver and kidney of male Wister rats) as compared to the products obtained by physical mixing or co precipitation methods. **Ali H. Al-Marzouqi., *et al* (2007)** [65]

9. Solid dispersion containing pellets of Itraconazole for enhanced drug dissolution rate. The influence of process parameters used during high shear pelletization on the pellet properties including pellet size and dissolution rate was also studied. Solid dispersions of Itraconazole were prepared with Eudragit® E100, a hydrophilic polymer, by a simple fusion method followed by powdered and characterized by differential scanning calorimetry and X-ray powder diffraction. Solid dispersions containing pellets were consequently prepared using a lab-scale high shear mixer. In order to improve the product quality, a central composite design was applied to optimize the critical process variables, such as impeller speed and kneading time, and the results were modeled statistically. Itraconazole was presented as an amorphous state in the solid dispersion prepared at a drug to polymer ratio of 1:2. Both studied parameters had great effect on the responses. Powdered solid dispersion and pellets prepared using the optimal parameter settings showed approximately 30- and 70-fold increases in dissolution rate over the pure drug, respectively. Solid dispersion prepared by simple fusion method could be an option for Itraconazole solubility enhancement. Pelletization process in high shear mixer

can be optimized effectively by central composite design. **Siling Wang., *et al* (2007)** [66]

10. Investigated the feasibility of a supercritical fluid process, called aerosol solvent extraction system (ASES), for producing solid-state inclusion complexes of itraconazole (ITR) with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). ITR was complexed with HP-β-CD at temperatures of 35–55 ◦C, pressures of 83–140 bar, CO2 densities of 0.498–0.801 g/cm3, and solution concentrations of 1–5% (w/v). The ASES-processed inclusion complex powders were observed to consist of agglomerates of very fine (100–500 nm) particles. From the experimental results of X-ray diffraction and differential scanning calorimetry, it was found that ITR intermolecular interacted with the HP-β-CD cavity, resulting in the formation of inclusion complex. Furthermore, the ASES-processed ITR/HP-β-CD powders showed a significant enhancement in the ITR solubility (up to 753.6 μg/mL) in an aqueous medium of pH 1.2. The aqueous solubility of ITR increased with pressure at a constant temperature, and we could obtain a relatively high solubility of 341 μg/mL at 140 bar and 35 ◦C. In a solution concentration range of 1–5% (w/v), the solubility increased with decreasing concentration, yielding 289–407 μg/mL. When the molar ratio of ITR to HP-β-CD was varied from 1:1 to 1:3, the ITR solubility increased with HP-β-CD content, giving a value of 753.6 μg/mL for the 1:3 ratio. For the ASES-processed ITR/HP-β-CD powders, the percent dissolution of ITR also increased considerably and about 90% of ITR was dissolved within 5–10 min. **Jong-Hoon Ryua,. *et al:* (2008)**

11. Domperidone is a widely used antiemetic, poorly water soluble drug, erratically absorbed in stomach and possess several dissolution-related problems thus it has poor bioavailability. Solubility of a drug plays a very important role in dissolution and hence absorption of drug which ultimately affects its bioavailability. Hence, by considering the facts related to drug, attempts have been made to formulate inclusion complexes using methylated β-cyclodextrin and also to study the effect of preparation method. Inclusion complexes were prepared using methylated β-cyclodextrin in 1:1 and 1:2 molar ratios. Kneading, ultrasonification and physical

mixture method were used for preparation of inclusion complexes. All the inclusion complexes were characterized using FTIR, DSC and XRD. The solubility and dissolution results revealed that there was a considerable increase in solubility and dissolution of all inclusion complexes as compared to pure drug. It was highest in case of methylated β-cyclodextrin in 1:1 molar ratio using ultrasonification method (USM1). Stability study revealed that all complexes were stable for a period of three months. **Dhananjay S Ghodkea., *et al:* (2008)** [68]

12. Investigated the influence of temperature and moisture, polymer blends of polyethylene glycol 6000 (PEG 6000) and hydroxypropylmethylcellulose 2910 E5 (HPMC 2910 E5) and solid dispersions of itraconazole in these polymer blends were spray dried, further dried for 2 weeks and stored at three different conditions: 25ºC, 0% relative humidity (RH); 25ºC, 52% RH; 60ºC, 0% RH. MTDSC analysis of the polymer blends revealed that at 25ºC, 52% RH, PEG 6000 recrystallized to a high extent. At 60ºC, 0% RH the two polymers were miscible, probably due to the removal of bound water. In the ternary dispersions the polymers behaved similarly. The crystallinity degree of itraconazole in samples stored at 25ºC, 52% RH and at 60ºC, 0% RH was increased compared to the samples stored at 25ºC, 0% RH, probably due to the plasticizing effect of moisture at 25ºC, 52% RH and to an increased mobility at 60ºC, 0% RH. XPS analysis revealed a redistribution of itraconazole at the surface as Itraconazole recrystallized from the blend. Dissolution tests revealed that a decrease in the itraconazole release was directly related to its crystallinity degree, no correlation was found with the crystallinity degree of PEG 6000. **Guy Van den Mooter., *et.al:* (2008)**

13. Solid dispersions was prepared by co-spray-drying of TPGS-stabilized Itraconazole Nanosuspension with Aerosil®200, followed by heat treatment of the powders. The Itraconazole/Aerosil®200 weight ratios amounted to 50/50, 30/70, 40/60 and 20/80. The Itraconazole content of the powders was close to the expected value, with relative errors between 0.3% and 7.8%. X-ray powder diffraction (XRPD), solid state NMR (SSNMR) and differential scanning calorimetric (DSC) evaluation on

the powders revealed the formation of amorphous Itraconazole and the absence of glassy Itraconazole. Dissolution of the powders was enhanced compared to crystalline and glassy Itraconazole (a 2-dimensional structured form of Itraconazole). However, no clear trend could be observed between drug loading and dissolution performance of the solid dispersions. Upon storage, conversion to crystalline Itraconazole was observed for the 50/50 powder based on XRPD, SSNMR and DSC measurements. Although the 40/60 powder remained X-ray amorphous upon storage, DSC did reveal that a small fraction (7.5±1.6% after 10 months of storage) of Itraconazole crystallized upon storage. For the 30/70 and 20/80 dispersions, no crystallization could be seen. After 10 months of storage, an important change in the dissolution behavior of the powders was observed. A decrease in dissolution performance was seen for the 50/50 dispersion, which could be attributed to the crystallization of Itraconazole. On the other hand, the 40/60, 30/70 and 20/80 dispersions showed an increase in dissolution rate (more than 60% after 10 min). Although not completely clear at this stage, adsorption of Itraconazole onto the Aerosil®200 surface during storage might be responsible for this behavior. Finally, in vivo experiments were performed in the rat. Oral bioavailability of the 30/70 dispersion was, although lower compared to the marketed Sporanox® formulation, significantly enhanced compared to the crystalline drug. **Guy van den mooter., *et al;* 2009.**[69]

14. Improved dissolution and in vivo bioavailability of Itraconazole prepared by the solid dispersion using lipid materials as inert excipients. The drug encapsulated dispersions are spray dried and are duly characterized for drug content, particle size distribution, drug morphological conversion, drug compatibility with the excipients, *in vitro* dissolution and *in vivo* bioavailability. The drug content in the prepared dispersions is between 80.0% and 95.0% (w/w) of the theoretical values and the mean volume diameter (VMD) of the particles collected from drying chamber and cyclone are found to be 21.65 & 33.66µm, respectively. The DSC thermo grams have indicated the morphological conversion of Itraconazole to amorphous form. The saturation solubility for Itraconazole in the spray dried formulation is 5.2 and 2.6 times higher to the plain drug and spray dried drug, respectively. The dissolution

of Itraconazole in acetate buffer pH 1.2 is 69% in the solid dispersion formulation where as it is only 17% in the plain drug in 60 min. And the enhancement of dissolution is 4.07 times higher to plain drug after 60mins. The formulations have demonstrated the significant improvement of bioavailability (AUC=14384ng/h/ml) compared to plain drug suspension (AUC=4384ng/h/ml). These results demonstrated the efficacy of solid lipid dispersions for the enhancement of oral bioavailability of Itraconazole by increasing its aqueous solubility**. R.S Prasad., *et al;* (2010)** [70]

BACKGROUND OF THE STUDY

Itraconazole is a poorly soluble antifungal agent and so its bioavailability remains problematic solid dispersion approach using different inert carriers and inclusion

complex with β- cyclodextrins have been investigated and an improved dissolution and bioavailability of Itraconazole reported. Itraconazole is a weak base and so absorption of drug is influenced by physiological pH of the environment to which it is exposed. Itraconazole absorption is improved by the presence of food in the stomach due to increase in pH, previous study showed an improved dissolution and pharmacokinetics of Itraconazole β- cyclodextrin inclusion complex in gastric fluids in animal model and the results compared between inclusion complex prepared by physical mixture, conventional co-precipitation method and super critical carbon dioxide (SC CO_2). However the influence of different pH conditions of the stomach on the dissolution and bioavailability of Itraconazole - β-cyclodextrin inclusion complex prepared by conventional co-precipitation method is lacking and hence the present study to investigate the same.

CHAPTER - III

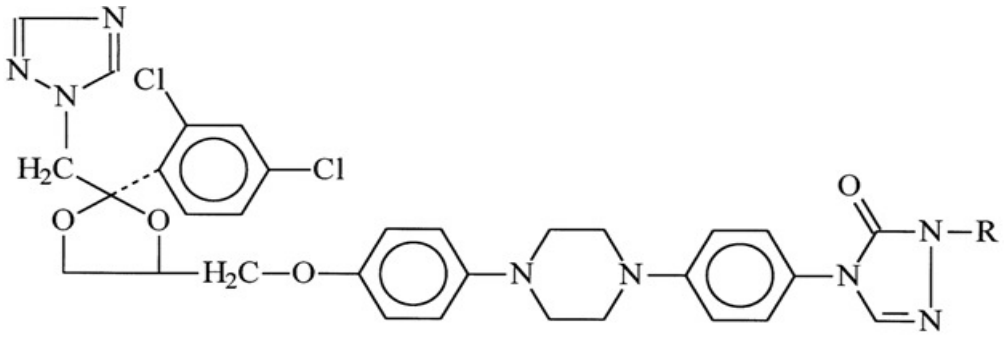
PROFILES

3.1 DRUG PROFILE

Itraconazole is an orally active triazole anti-fungal agent.[71] Itraconazole, which was first synthesized in 1980, Itraconazole having broad spectrum of activity against a number of fungal species like histoplasmosis, blastomycosis and onychomycosis.[72] including Dermatophytes, Such as *Malassesia fur-fur*, *Candida* species, *Aspergillus* species, and *Histoplasma capsulatum* var. *capsulatum.*

Itraconazole is an extremely weak base (P^{Ka} = 3.7) which is virtually unionized at physiological P^{H}.[73,74] it is also very lipophilic with Octanol /water log partition coefficient of 5.66 at a P^{H} of 8.1. The drug has a P^{H} dependent Dissolution resulting in low and variable oral absorption. The absorption of the drug is enhanced by the concurrent administration of food.[75] Based on the Biopharmaceuticals classification system, itraconazole is an example of class II compounds.[39]Meaning that its oral bioavailability of the drug is dissolution rate limited.

Chemical Structure: [76]



R = CH(CH3)—CH2—CH3 : Itraconazole

CHEMICAL NAME: [76]

(cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one)

Molecular formula : $C_{35}H_{38}Cl_2N_8O_4$

Molecular weight : 705.64

Melting point : 166°c to 170°c

Half life : 21hours

Protein binding : 99%

Bioavailability : 55%

Synonym : Sporanox, Itrizole, Fungitra, Triasporm, Sopranos, Oriconazole

Physicochemical properties:

Itraconazole is almost white to half white powder. Partially in-soluble in water, and freely soluble in methylene di-chloride, sparingly soluble in Tetrahydrofuran, very slightly soluble in the alcohol.

Mechanism of action: [76]

The triazoles have a similar mechanism of action to that of the imidazoles. The free azole nitrogen competes for oxygen at the catalytic heme iron atom of cytochrome P-450 enzymes. Inhibition of cytochrome P-450 enzymes prevents the synthesis of ergosterol in fungal cell membranes by limiting the C-l4 demethylation of lanosterol,

which is critical for the synthesis of ergosterol. Lack of ergosterol alters the membrane fluidity and steric relationships of other membrane-associated enzymes and also results in an accumulation of phospholipids and unsaturated fatty acids within fungal cells. Itraconazole binds only weakly to mammalian cytochrome P-450 and it has a much higher affinity than ketoconazole for fungal P-450 enzymes.

Pharmacokinetics:

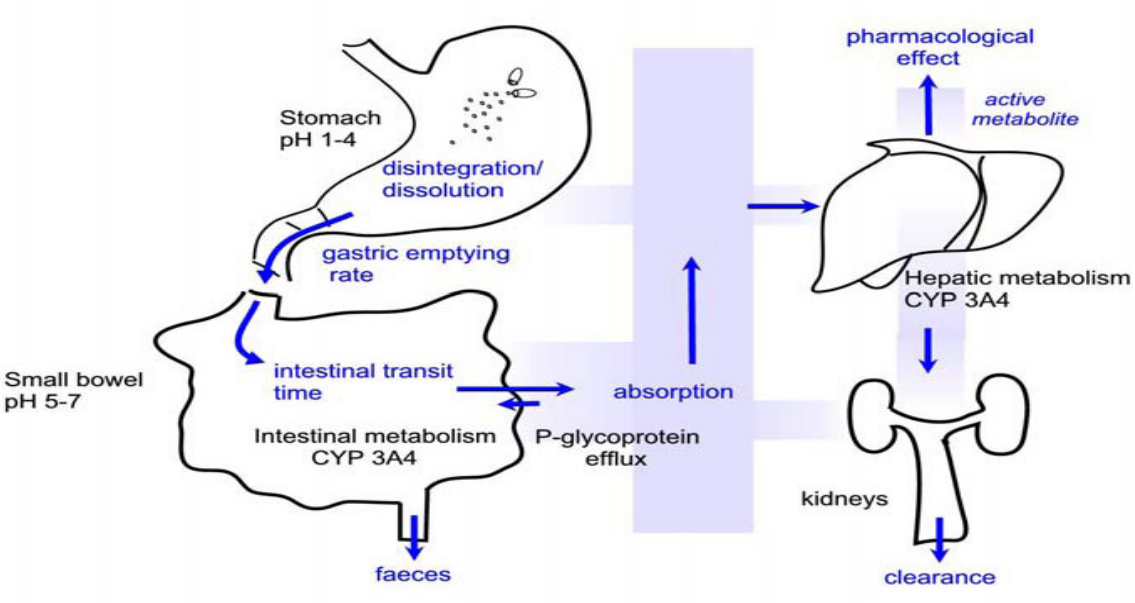


Fig. 1: Pharmacokinetic absorption of Itraconazole

The drug is absorbed in to the systemic circulation and it is highly bound to the plasma proteins. Plasma concentration of the drug is low. The drug is extensively metabolized by the liver by cytochrome P450 3A4 iso-enzyme (CYP3A4) which results in production of both active and inactive metabolites. Hydroxy metabolites are active[77] Dissolution rate of itraconazole is optimal at a pH 1-4, impaired absorption occurring above these pH values. Gastric emptying rate also plays an important role in absorption of itraconazole.[78] Absorption of drug is influenced by the food. Food increases the absorption the drug. The active and in active metabolites are clear by the kidney half of

the drug under go by the first pass metabolism.[79] Unchanged drug is excreted by the feces (3-18%).

Metabolism[76]

The main metabolic Path ways are oxidative scission of the dioxolane ring, oxidative degradation of the piperazine ring and aliphatic oxidation and 'N-dealkylation at the l-methylpropyl substituent.

Therapeutic uses:

It is used in the treatment for several fungal infections, commonly used to treat Toenalis. Effective in treating pulmonary infections like blastomycosis and histoplasmosis. It is active against. Dermatophytes, *Malassesia fur - fur*, *Candida* species, *Aspergillus* species, and *Histoplasma capsulatum* var. *capsulatum.* it is used in the treatment of the veginal candidacies. It is also a first line treatment in many types of fungal infections like skin, nails, mucocutaneous and oropharyngel infections.

Dosage:

In treating deep mycoses, a loading dose of 200mg of itraconazole is administered three times daily for the first three days. For maintenance therapy two 100mg capsules are twice daily with food.

HIV: Maintenance HIV infected patients disseminated histoplasmosis 200mg daily is used.

Onchy-mycosis can be treated with 200mg once daily for 12 weeks or 200mg twice daily for one week out of each month so called pulse therapy.

Vulvovaginai 200 mg twice daily 1 day

Candidiasis or 200 mg daily 3 days

Pityriasis versicolor 200 mg daily 7 days
Dermatophytosis 100 mg daily 15 days

Oral candidiasis 100 mg daily

Fungal keratitis 200 mg daily

Highly keratinized regions as in plantar tine a pedis and palmar tine a manus require an additional treatment of 15 days at 100 mg daily "Dose to be double in case of immune suppression.15 days 21 days.

Drug interactions: [76]

Enzyme-inducing drugs such as rifampicin and phenytoin significantly reduce the oral bioavailability of itraconazole. Consequently, monitoring of itraconazole plasma concentrations is advised when enzyme-inducing agents are co-administered[43]. Itraconazole can inhibit the metabolism of drugs metabolized by the cytochrome 3A family resulting in an increase and or a prolongation of their effects, including side-effects.

Examples are:

Terfenadine, Astemizole, Cisapride, Oral midazolam and triazolam. These agents should not be used in patients treated with itraconazole. If midazolam is administered intravenously, special precautions are required since the sedative effect may be prolonged.[80-86]

Warfarin, Digoxin, Cyclosporine-A and possibly tacrolimus. The dosage of these drugs, if co-administered with itraconazole, should be reduced if necessary. [87]

3.2 β -CYCLODEXTRIN

Cyclodextrins are cyclic oligosaccharides containing at least 6 D-(+) glucopyranose units attached by α-(1, 4) Glucosidic bonds. (CDs), They were first discovered in 1891.[25] The outside surface of these molecules is hydrophilic and the inside surface hydrophobic, They are able to include, fully or partially, in their cavity large organic molecules by non-covalent interaction.[88-89] (hydrogen bonds, Vander-Wall forces).

Among the various approaches that have been used to improve the solubility and dissolution rate of drugs, complexation with cyclodextrins is one of the most promising ones. Which enhance their solubility, dissolution rate, chemical stability and bioavailability and reduce their side effects and toxicity. [90]

Conventional methods for the preparation of solid inclusion complexes between cyclodextrins and various drugs includes kneading, co-evaporation, sealed-heating, co-grinding, spray-drying and freeze-drying.[91] The use of supercritical carbon dioxide (sc co_2) has been recently proposed for the preparation of various drug-cyclodextrin inclusion complexes for enhanced solubility and dissolution rate.[92,93]

Cyclodextrins are of three types: α, β, and, γ. Cyclodextrins, referred to as first generation or parent cyclodextrins. α, β, and, cyclodextrins are composed of six, seven and eight α-(1,4)-linked glycosyl units, respectively[94] β-Cyclodextrin is the most accessible, the lowest-priced and generally the most useful. Cyclodextrins is able to improve drug delivery through biological membranes. The cyclodextrin are high molecular weight compounds ranging from (1000 to 1500).There are numerous applications for cyclodextrins in the pharmaceuticals field. For example, the addition of α or β -cyclodextrin increases the water solubility of several poorly water-soluble substances, and improve oral bioavailability, β- CD has been widely used in the early stages of pharmaceutical applications because of its readily availability and cavity size suitable for the widest range of drugs. But the low aqueous solubility and nephro toxicity limited the use of β-CD especially in parenteraldrug delivery. Method of preparation by co-grinding, kneading, solvent evaporation, co-precipitation, spray drying, or freeze

drying can affect drug/CD complexation. The effectiveness of a method depends on the nature of the drug and CD.[95, 96, 97] In many cases, spray drying, [98-99] and freeze drying.[100] were found to be most effective for drug complexation.

Synonym : Beta – cyclodextrin, βCD, BCD, β-Sachardinger

Dextrin cyclodextrin B, cycloamyloses,
Cyclomaltoses and Schardinger dextrins

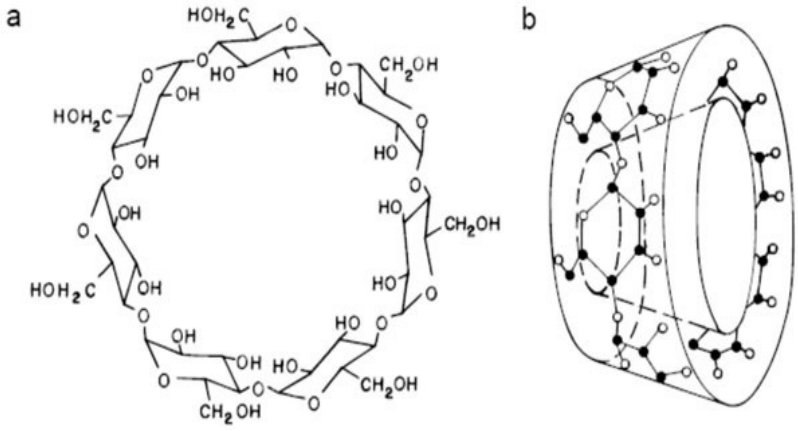
Chemical formula : $(C_6H_{10}O_5)_7$

Molecular weight : 1135.00

Solubility : Sparingly soluble in water, Freely soluble in hot

Water Slightly soluble in alcohol

Structure :[95]



The chemical structure (A) and the toroidal shape (B) of the cyclodextrin molecule

Table.3: Some characteristics of (α, β, γ, δ).

Type of CD	Cavity diameter A	Molecular weight	Solubility(g/100ml)

α – CD	4.7 – 5.3	972	14.5
β - CD	6.0 – 6.52	1135	1.85
γ - CD	7.5 – 8.3	1297	23.2
δ - CD	10.3 -11.2	1459	8.19

Mechanism of formation of Inclusion complexes:

Cyclodextrins are able to form solid inclusion complexes (host–guest complexes) with a wide range of solid, liquid and gaseous compounds by a molecular complexation. [103] in these complexes, a guest molecule is held within the cavity of the cyclodextrin host molecule. Complex formation is a dimensional between host cavity and guest molecule [104] The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes.[105] No covalent bonds are broken or formed during formation of the inclusion complex.[106] Complexes can be formed either in solution or in the crystalline state and water is typically the solvent of choice. Inclusion complexation can be accomplished in a co-solvent system and in the presence of any non-aqueous solvent.

Drug + CD = Drug: CD Complex

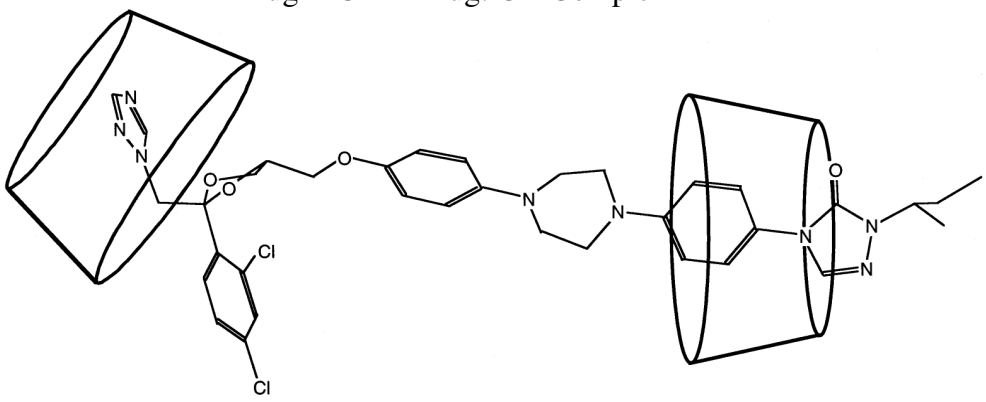


Fig 2: Itraconazole and beta cyclodextrin inclusion complex.

Applications of cyclodextrins

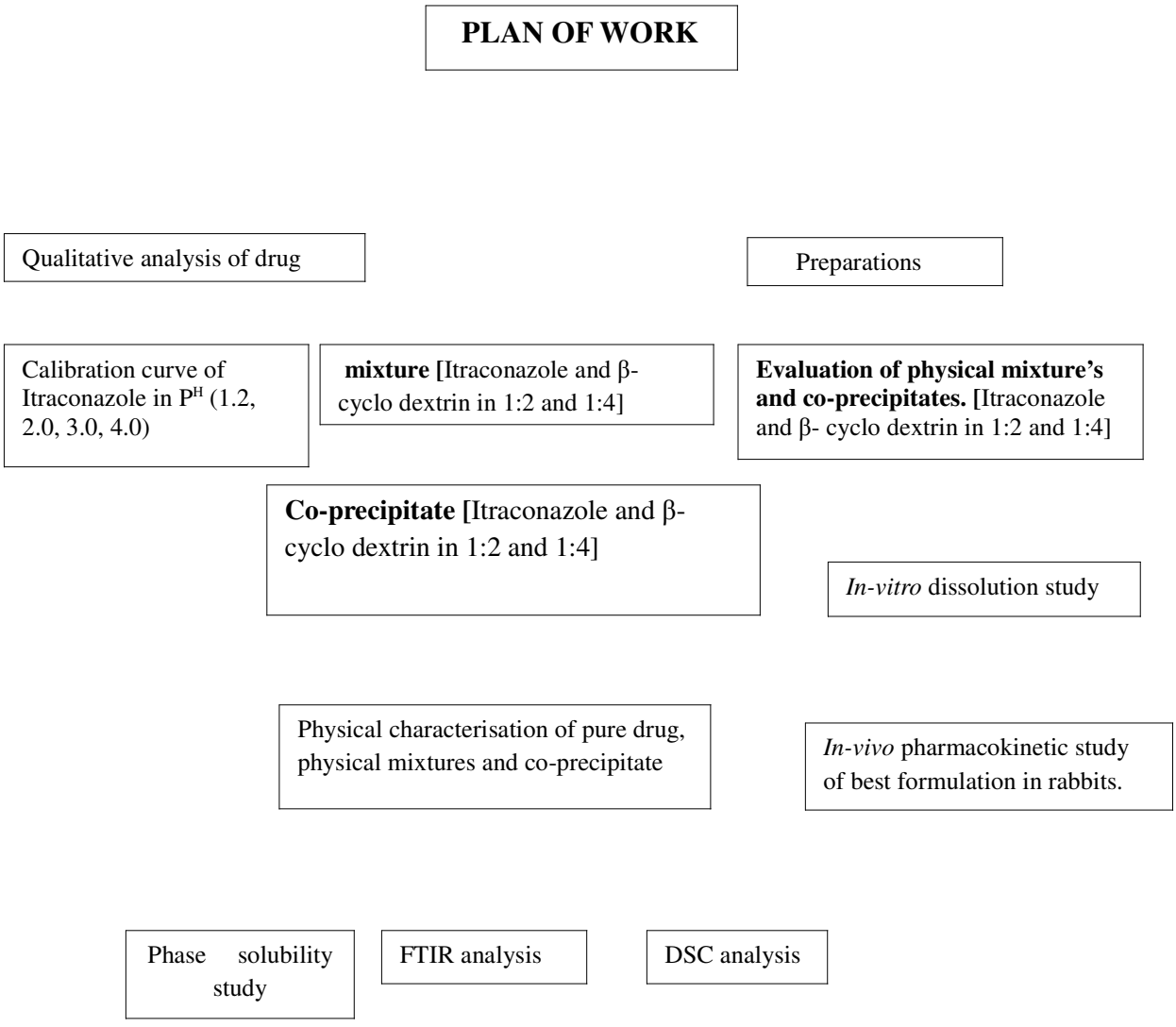
Since each guest molecule is individually surrounded by a cyclodextrin (derivative) the molecule is micro-encapsulated from a microscopically point of view. This can lead to advantageous changes in the chemical and physical properties of the guest molecules.

i. Stabilization of light or oxygen-sensitive substances.
ii. Modification of the chemical reactivity of guest molecules.
iii. Fixation of very volatile substances.
iv. Improvement of solubility of substances.
v. Modification of liquid substances to powders.
vi. Protection against degradation of substances by microorganisms.
vii. Masking of ill smell and taste.
viii. Masking pigments or the color of substances.

CHAPTER - IV

PLAN OF WORK

4. PLAN OF WORK



CHAPTER - V

AIM &OBJECTIVES

5. AIM AND OBJECTIVE

AIM: To investigate dissolution and *in–vivo* bioavailability of itraconazole β – cyclodextrin complex.

OBJECTIVE: The following studies were performed.

- ❖ Phase solubility study.
- ❖ FTIR spectra.
- ❖ Differential scanning calorimetric.
- ❖ *In-vitro* dissolution.
- ❖ *In-vivo* pharmacokinetic study.

CHAPTER -VI

MATERIALS

6. Materials

S.no	Drugs and chemicals	Manufacturer/ supplier
1.	Itraconazole usp	Hetero Pvt. Ltd
2.	β- cyclodextrin	Hi-media laboratories Pvt.Ltd
3.	Methanol	Loba Chemie Pvt.Ltd.
4.	Tragacanth	Loba Chemie Pvt.Ltd.
5.	Alcohol	Loba Chemie Pvt.Ltd.

Table.4

S.no	Drugs and chemicals	Manufacturer/ supplier
1.	UV-Spectrophotometer	Perkin Elmer
2.	Infra-Red Spectrophotometer	Perkin Elmer spectrum RX1 FT-IR
3.	Differential Scanning Calorimetry	Schimadzu, DSC 60.
4.	Weighing Balance	Schimadzu

Table .5

CHAPTER - VII

METHODOLOGY

7. METHODOLOGY

7.1 Procedure for calibration curve of Itraonazole

Preparation of Calibration Curve.

Itraconazole content was estimated by measuring the absorbance at 265nm. The standard curve for Itraconazole was prepared by using different pH [1.2, 2.0, 3.0, 4.0,]

Procedure

100mg of the Itraconazole was dissolved in different pH [1.2, 2.0, 3.0, 4.0,] and shaken well and sonicated for 5min to get a clear solution and make up the volume to 100ml with the buffer. From the stock solution take 10 ml and make up the volume to 100ml. From the working standard take 2, 4, 6, 8, 10 ml and diluted to 10 ml to get the concentrations of 2, 4, 6, 8, 10(µg/ml) Graphs was Plotted by taking time on X- axis and concentration on Y- axis.

7.2 Preparation of physical mixture

The physical mixtures was prepared by gently grinding the drug and cyclodextrin powders in a mortar in the ratio of 1:2 and 1:4 and they were pulverized and then mixed thoroughly in glass mortar and pestle until homogenous mixture is Obtained. Mixtures were passed through 0.45 µm sieve and used for further studies.

7.3 Preparation of the inclusion complexes by co- precipitation method

Required amount of β-cyclodextrin was dissolved in de-ionized water and a known amount of Itraconazole (100mg) (giving the desired drug to b-cyclodextrin molar ratio) was dissolved in methanol. The two solutions are heated to 65ºC and added to gather after completely dissolved. The final solution was then mixed continuously with a magnetic stirrer while heating at 65ºC and allowed, the organic solvent was allowed to evaporate and the mixture was cooled to 5ºC. The crystals were separated by filtration

through 0.45µm membrane filters. The sample was then dried and stored at room temperature.

7.4 *In- vitro* dissolution study

Dissolution profile of pure Itraconazole, physical mixture and solid dispersion were evaluated according to the method described in USFAD 900mL of the dissolution medium of different buffer pH (1.2, 2, 3, 4) was prepared with de-aerated water and placed in the vessel and temperature of medium was maintained at 37±0.5ºC. The sample was placed in the medium and the dissolution was performed at 100 rpm. 10 ml samples were withdrawn at 5, 10, 20, 30, 45, 60.min and equivalent amount of dissolution medium were added to maintain the sink condition. The obtained samples were filtered (0.45 µm pore size) and analyzed by Spectrophotometer at 265nm the experiments were carried out in triplicate and mean values and SD were recorded.

7.5. *In – vivo* bioavailability studies

Animals**:**

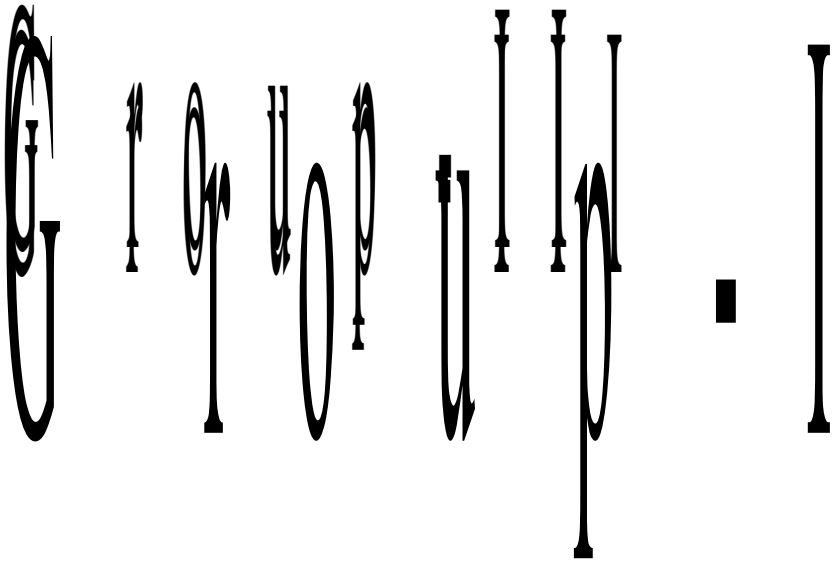
New Zealand white rabbits of either sex weighing 1.5 to 2.5kg were housed in animal house of Swamy Vivekanandha College of Pharmacy. The animals were fed with cabbage, and water. They were maintained in standard laboratory conditions 21±2 ^{0}C and relative humidity of 55-60%. The animals were overnight fasted before the experiment. The study protocol was approved by the Institutional Animal Ethical Committee and the protocol number is SVCP/IAEC/M.Pharm/03/2011.

Sex	:	Both Male and Female
Number of animals	**:**	9
Animal Dose	**:**	17.85mg Itraconazole sample.
		Itraconazole physical mixture in 1:4 (33.55mg)
		Itraconazole co- precipitation in 1:4 (30.7mg)

7.6. Procedure for collection of blood from marginal ear vein:

The animal was placed in restrainer. Smoothly shaved the hair of the ear with blade without disturbing the blood vessels. Ear was cleaned with 95% v/v alcohol on the collection site and rapid rubbing on the ear to dilate blood vessels which is easy to collect the blood. 24G needle was used to collect the blood from marginal ear vein. After collecting blood, clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding.

Experimental procedure**:** Rabbits were classified into 3 different groups each group consisting of 3 animals.



Each group of rabbits received the sample through an intra gastric tube. 1ml of blood was collected from each rabbit through marginal ear vein and placed in heparinized tubes at a time interval of 0, 0.5, 1, 1.5, 2, 4, and 8 hrs after sample administration and plasma was separated by using centrifugation at 4000 rpm and stored at -4°C, samples were analyzed by validated high performance liquid chromatography (HPLC).

7.7. Extraction of Itraconazole from plasma samples.

Blood sample was collected in tubes coated with EDTA. After sampling tubes are Centrifuged for 30 min at 4000 rpm at room temperature and the plasma was removed and stored at – 4°C until tested. Stability of the analytes under these conditions was verified. Three hundred micro litres of acetonitrile was added to the plasma and the tubes were thoroughly mixed on a vortex mixer. The tubes were centrifuged for 5 min, and the supernatant was analyzed by RP-HPLC. Itraconazole standards (DSM Pharma Chemicals Inc., South Haven, and MI) were prepared in plasma and treated identically to the samples. The assays were carried out using with an Alltech Altima C18 column, 250mm×4.6 mm, 5µm column. A mobile phase of 36% 5mM sodium phosphate (pH 6.7), 58% acetonitrile and 6% methanol was run isocratically at 37 °C at a flow rate of 1.1 ml/min. Analytes were detected by UV at 263 nm, with a bandwidth of 10 nm and fluorescence with 263 nm excitation and 380 nm emission. An injection volume of 100l was used.

7.8. Pharmacokinetic parameters

The pharmacokinetic parameters were calculated for each rabbit of Group - I, Group-II, Group - III by the semi logarithmic plot of plasma itraconazole concentration at various intervals. The following pharmacokinetic parameters were calculated:

Kinetic analysis of in-vitro release rates of itraconazole and β – cyclodextrin complex.

The results of in-vitro release profile were plotted in modes of data treatment as follows:

1. Zero – order kinetic model - cumulative % drug released versus time.

2. First – order kinetic model – log cumulative % drug remaining versus time.

a) Zero order kinetics:

Zero order release would be predicted by following equation.

$$A_t = A_0 - K_0 t$$

Where, A_t - Drug release at time 't'

A_0 - Initial drug concentration

K_0 - Zero-order constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear The data obeys zero order kinetics, with a slope equal to K_0.

b) First order kinetics:

First order release would be predicted by the following equation:

$$\frac{\text{Log } C_o - K_t}{\text{Log } C} = 2.303$$

Where,

C = Amount of drug remained at time 't'

C_o = Initial amount of drug

K = First order rate constant (hr^{-1})

When the data plotted as Log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

1. Elimination rate constant(K_e): The elimination rate constant was determined using the formula

$$K_e = -2.303 \times \text{slope of extrapolated curve}$$

2. Elimination half life($t_{1/2}$) : $t_{1/2}$ was calculated using the formula

$$T_{1/2} = 0.693/K_e$$

3. Absorption rate constant (K_a): This was determined by the method of residuals. The log linear portion of the decline phase was back extrapolated for each curve. The plasma concentration along this extrapolated line was C. the observed plasma concentration C was subtracted from the corresponding extrapolated value at each time point. The semi logarithmic plot of residuals (C-C) against time yields a straight line.

$$K_a = -2.303 \times \text{slope of residual line}$$

4. Absorption half life**:** It was calculated using the formula

$$T_{1/2(a)} = 0.693/K_a$$

5. Apparent volume of distribution(V_d):It was calculated using the formula

$$V_d = \frac{K_a F X_0}{(K_a - K_e) \text{ y intercept}}$$

6. Time to C_{max}(T_{max}):T_{max} was calculated using the formula

$$T_{max} = \frac{\ln K_a - \ln K_e}{K_a - K_e}$$

7. Maximum plasma concentration (C_{max}): C_{max} was calculated using the formula

$$C_{max} = \text{Y intercept } (e^{-Ke.\,Tmax} - e^{-Ka.Tmax})$$

8. Area under curve (AUC_{0-12}): AUC_{0-12} was calculated using the formula

$$AUC = \frac{F X_0}{V_d.K_e}$$

9. $AUC_{0-\infty}$ was calculated using the formula

$$AUC_{0-\infty} = \frac{C_0}{K_e}$$

7.9. STATISTICAL ANALYSIS:

The values are expressed in mean ± SEM. One way ANOVA followed by Tukey-Kramer multiple comparison tests.

CHAPTER - VIII

RESULTS

8. RESULTS

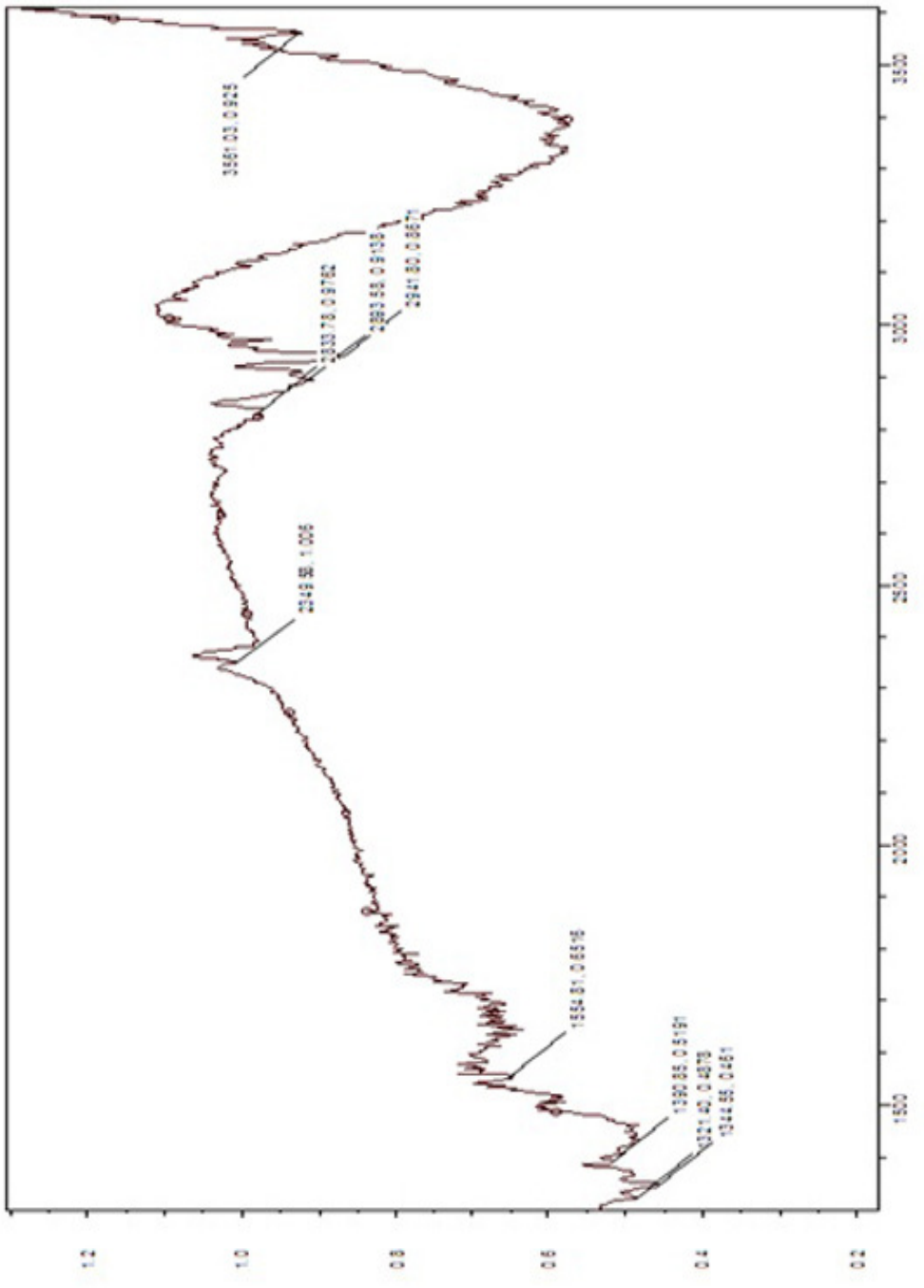
8.1 Compatability studies on Itraconazole and β- cyclo dextrin

The FTIR spectra of pure drugs, physical mixture and Co-precipitation of Itraconazole and β –cyclo dextrin are shown in the spectra exhibited presence of characteristic peaks of drugs in physical mixture indicating that there was no chemical interaction between the Itraconazole and β –cyclo dextrin.

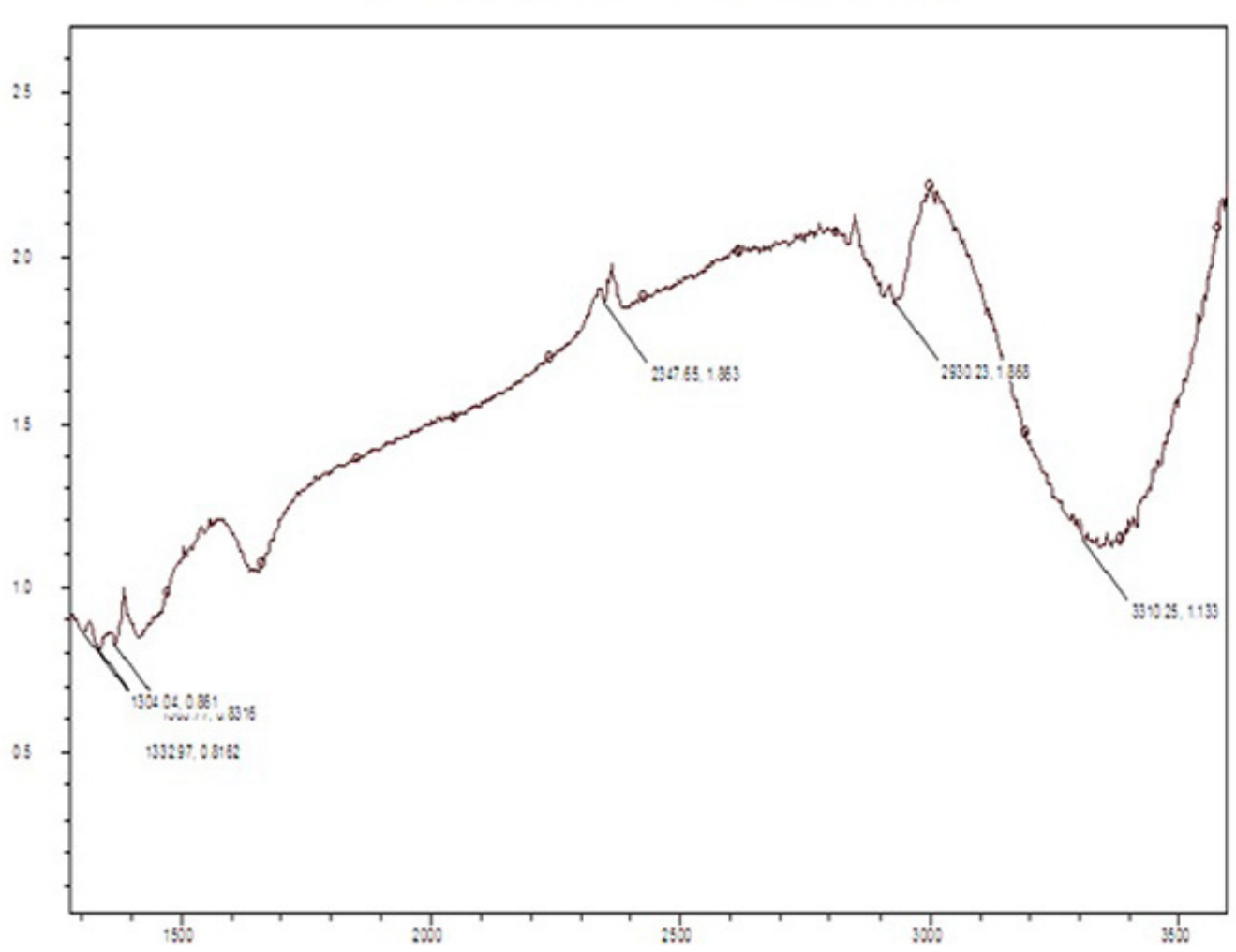
Table: 6 Comparative FTIR spectra of different formulations

Formulations									
S.no	**Groups**	**Pure ITC**	**Pure β-CD**	**Phy.mix 1:2**	**Phy.mix 1:4**	**Co-ppt1:2**	**Co-ppt1:4**	**Range**	**Property**
1.	C-H	+	+	+	+	_	_	2941.80	Bending aromatic
2.	N 3□-Nitrogen	+	_	+	+	+	+	3561.0	3□Nitrogen
3.	C-N 2□ Stretching	+	_	+	+	+	+	1554.85	2□Stretching
4.	C-CH3	+	_	+	+	_	_	1390.85	Alkane dimethyl
5.	α, β- 5 Member rings	+	_	+	+	_	_	1699.49	5 Member rings
6.	C-H bending	+	+	+	+	+	+	2893.70	Bending
7	OH 3□ Stretching	_	+	+	+	_	_	1304.04	Stretching
8	C-O Stretching	_	+	_	_	_	_	1332.97	Stretching

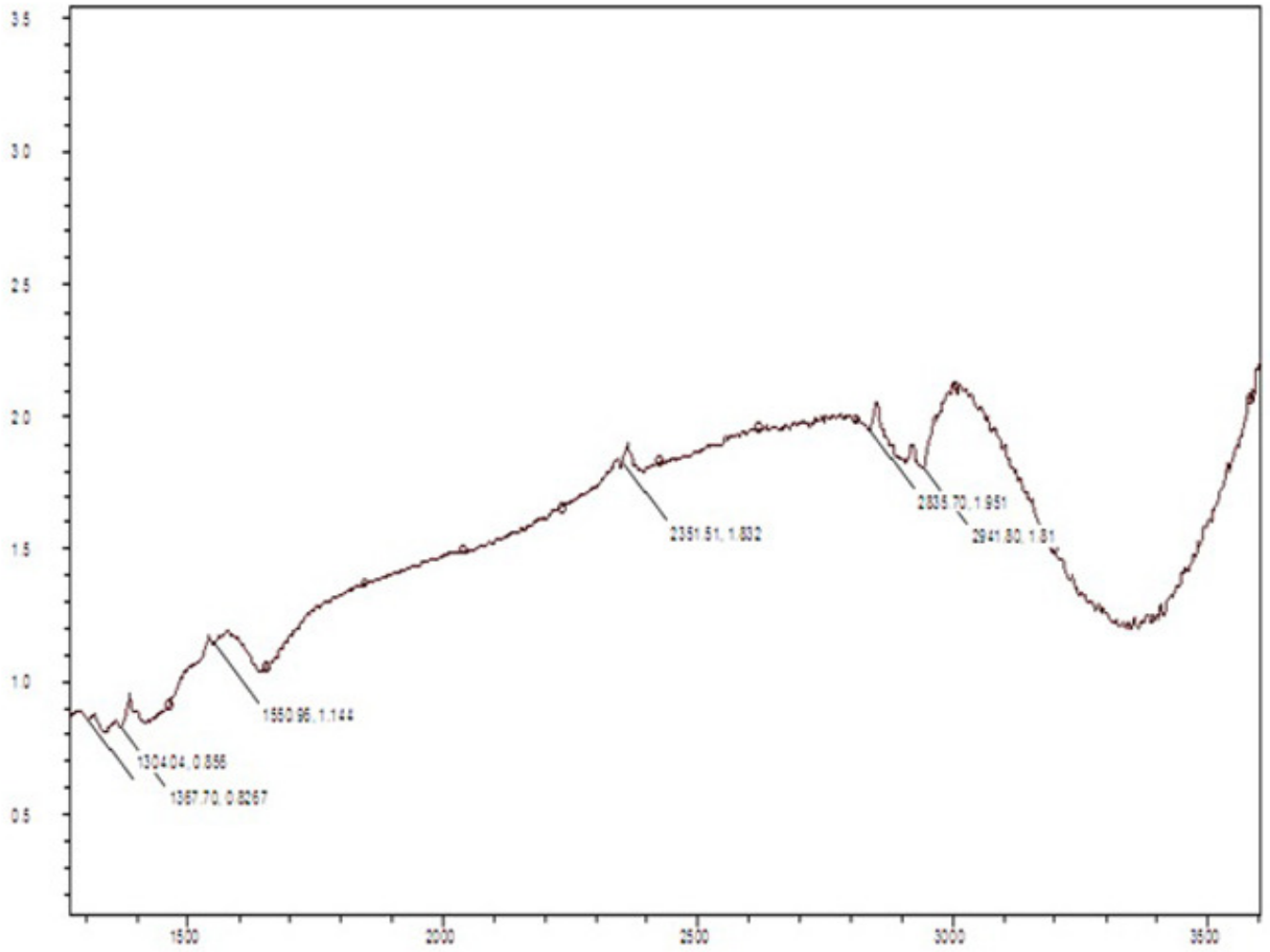
8.1 COMPATABILITY STUDIES ON ITRACONAZOLE AND β-CYCLO DEXTRIN



FTIR spectrum pure Itraconazole in functional group region

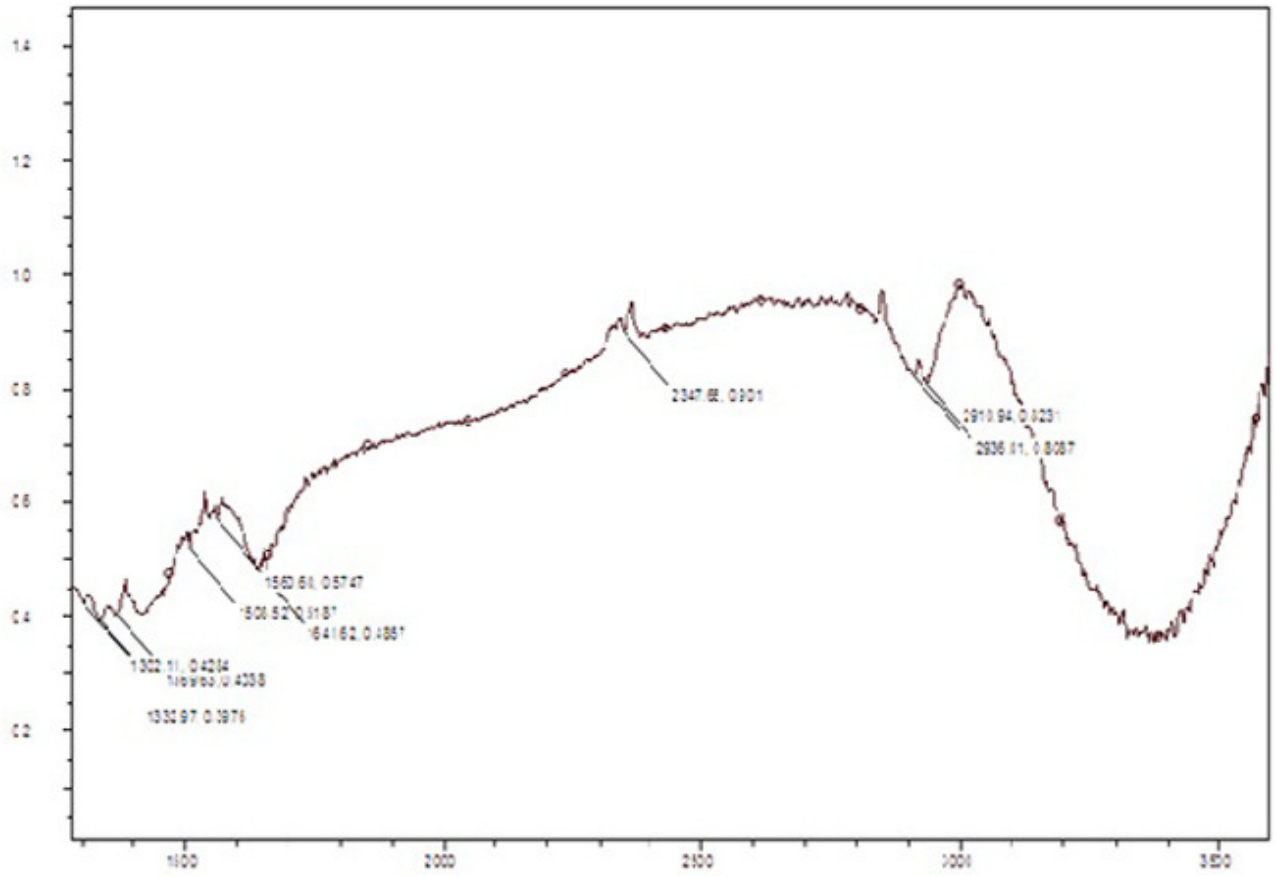


FTIR spectrum of pure β-cyclodextrin in functional group region

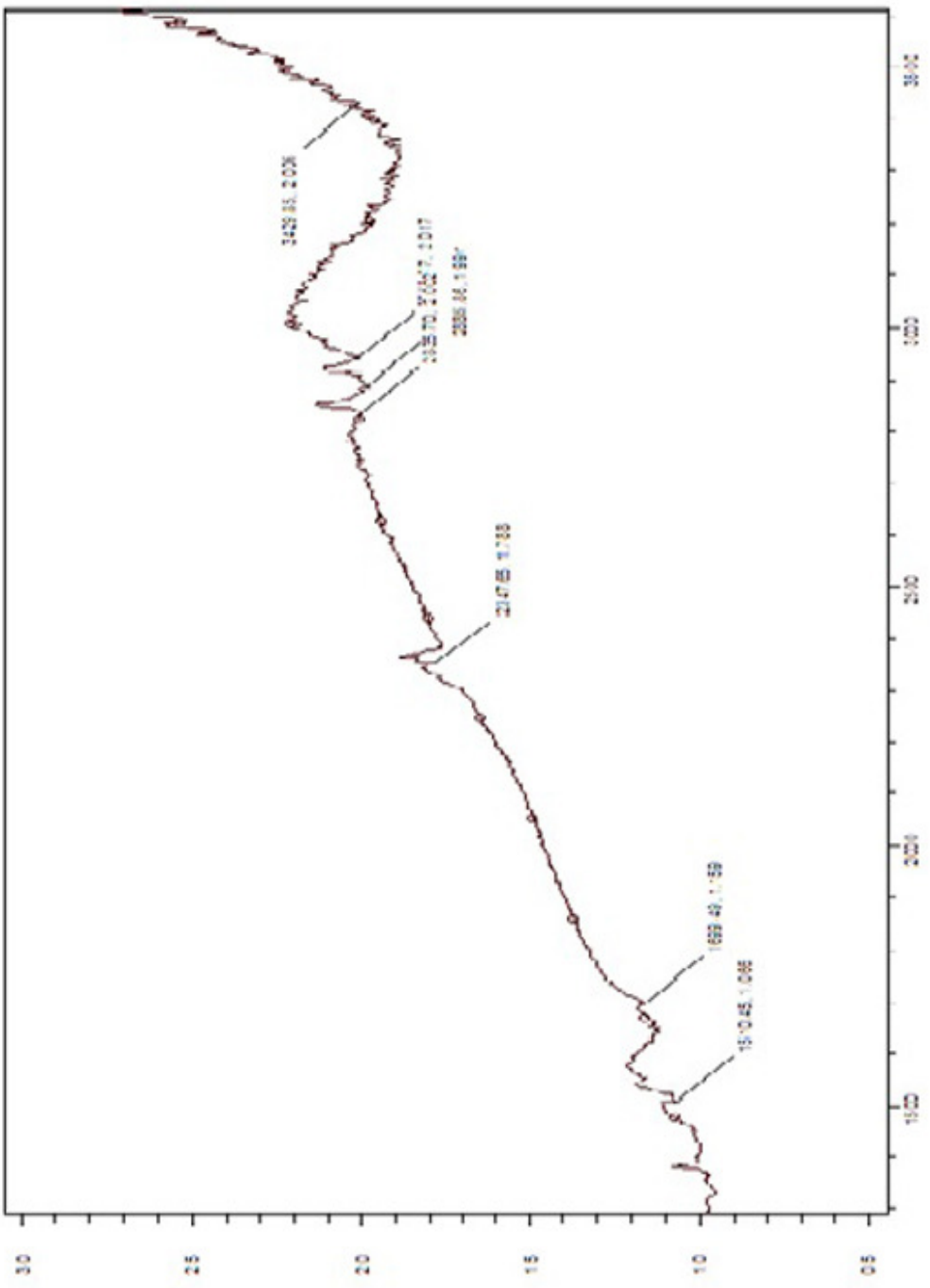


FTIR spectrum Physical mixture 1:2 in functional group region

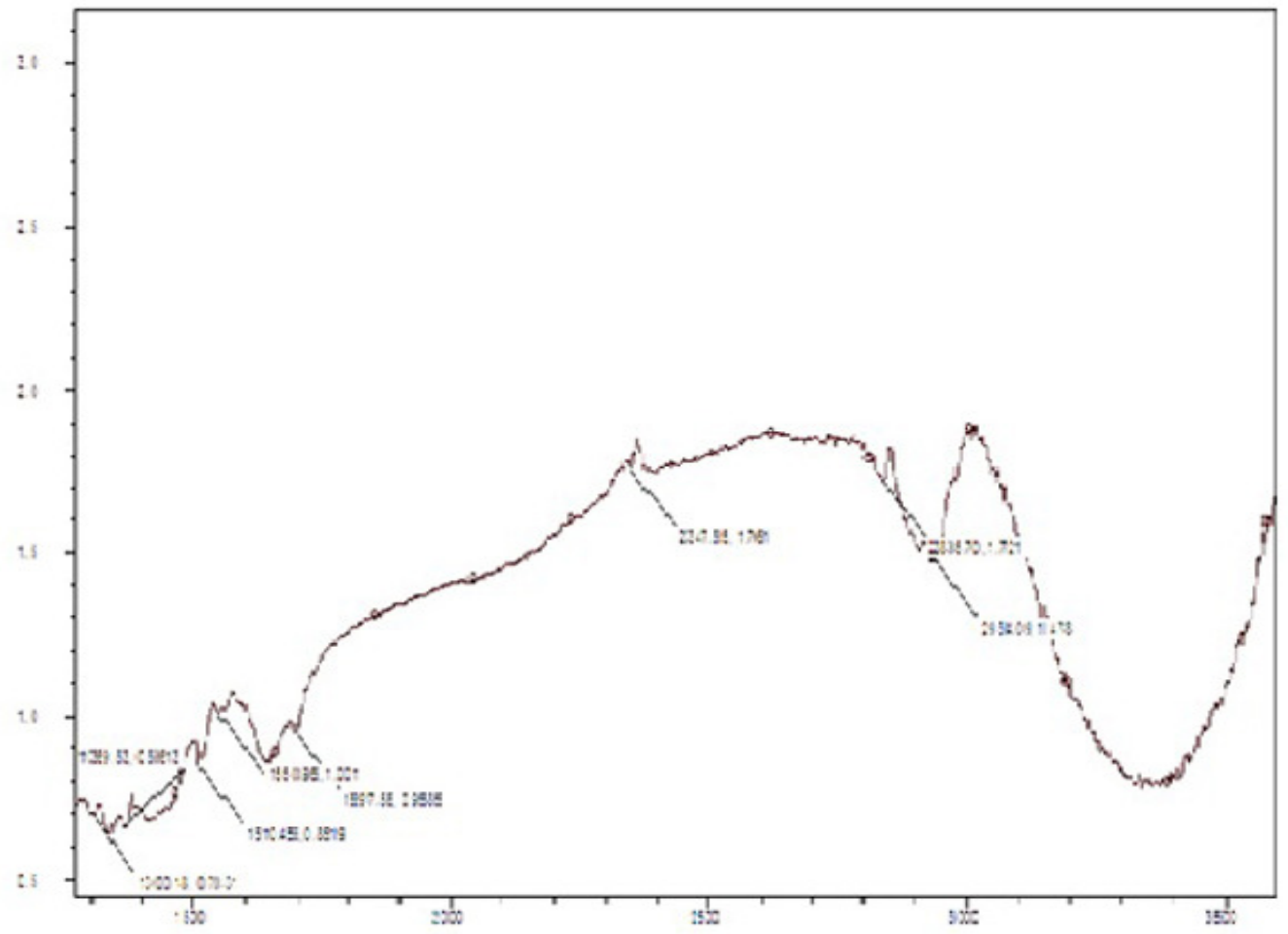
Figure. 5



FTIR spectrum Physical mixture 1:4 in functional group region



FTIR spectra of co-precipitation 1:2 in functional group region



FTIR spectrum co-precipitation 1:4 in functional group region

8.2 DSC PEAKS OF ITRACONAZOLE AND β – CYCLO DEXTRIN

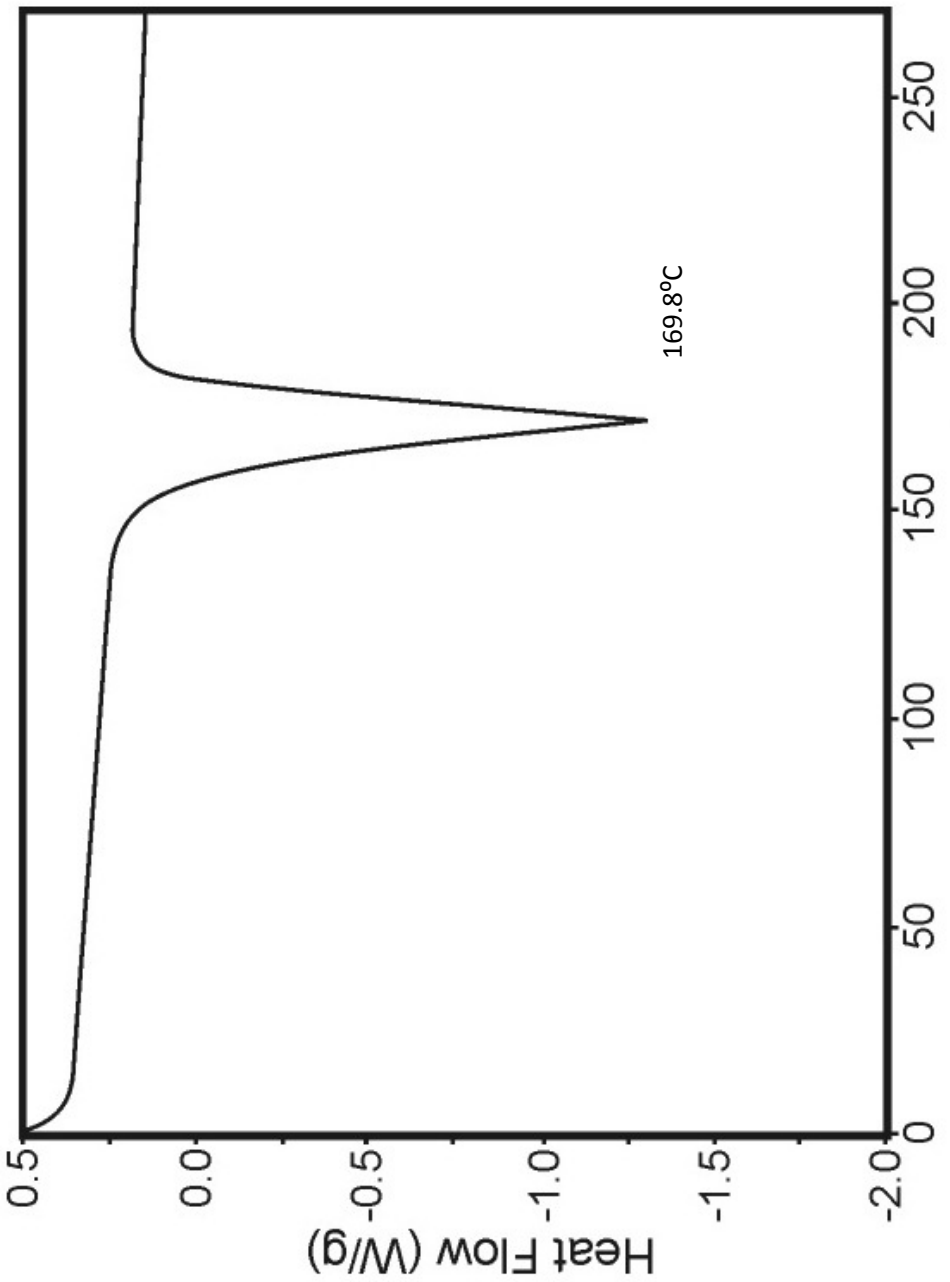
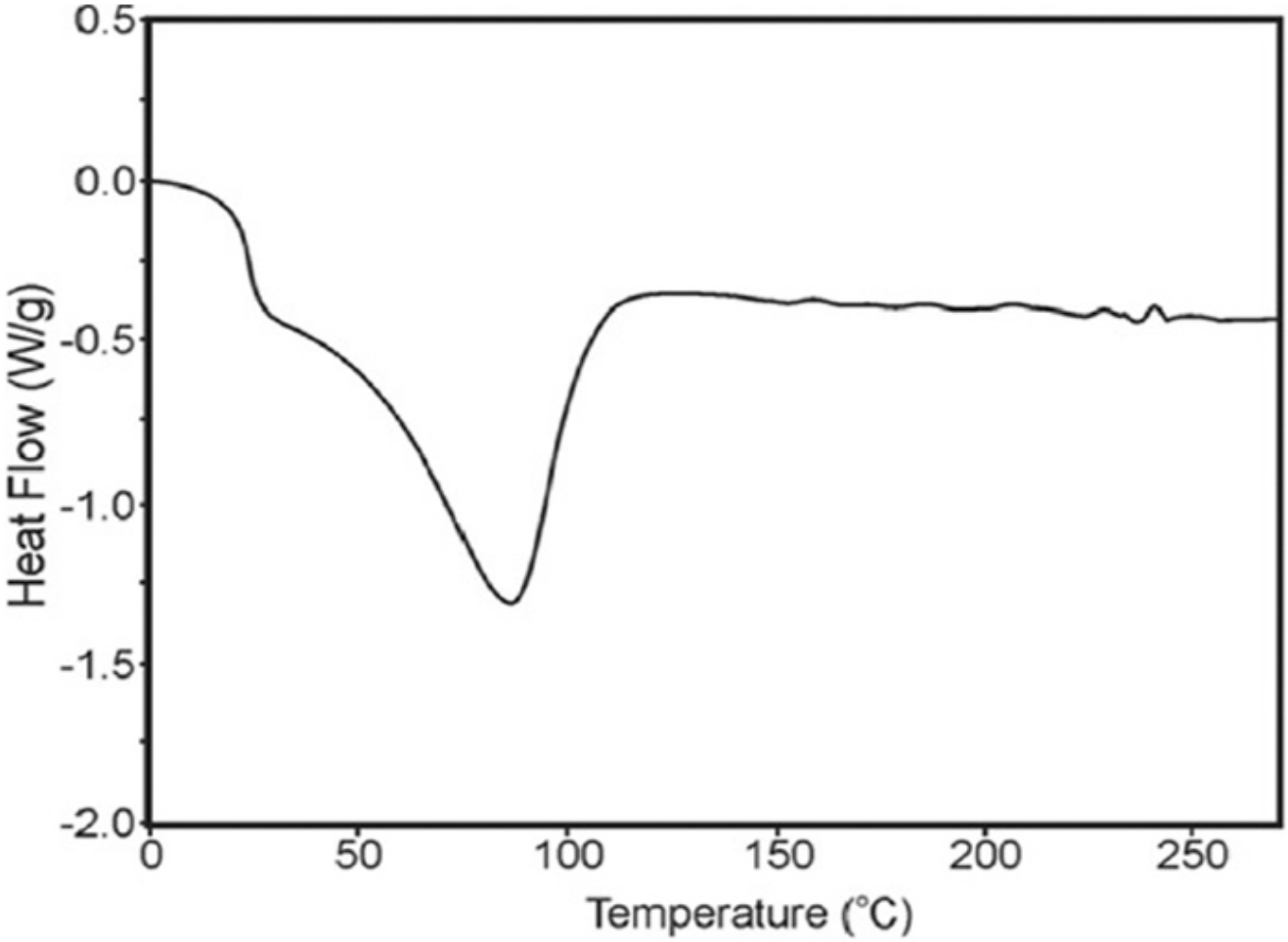
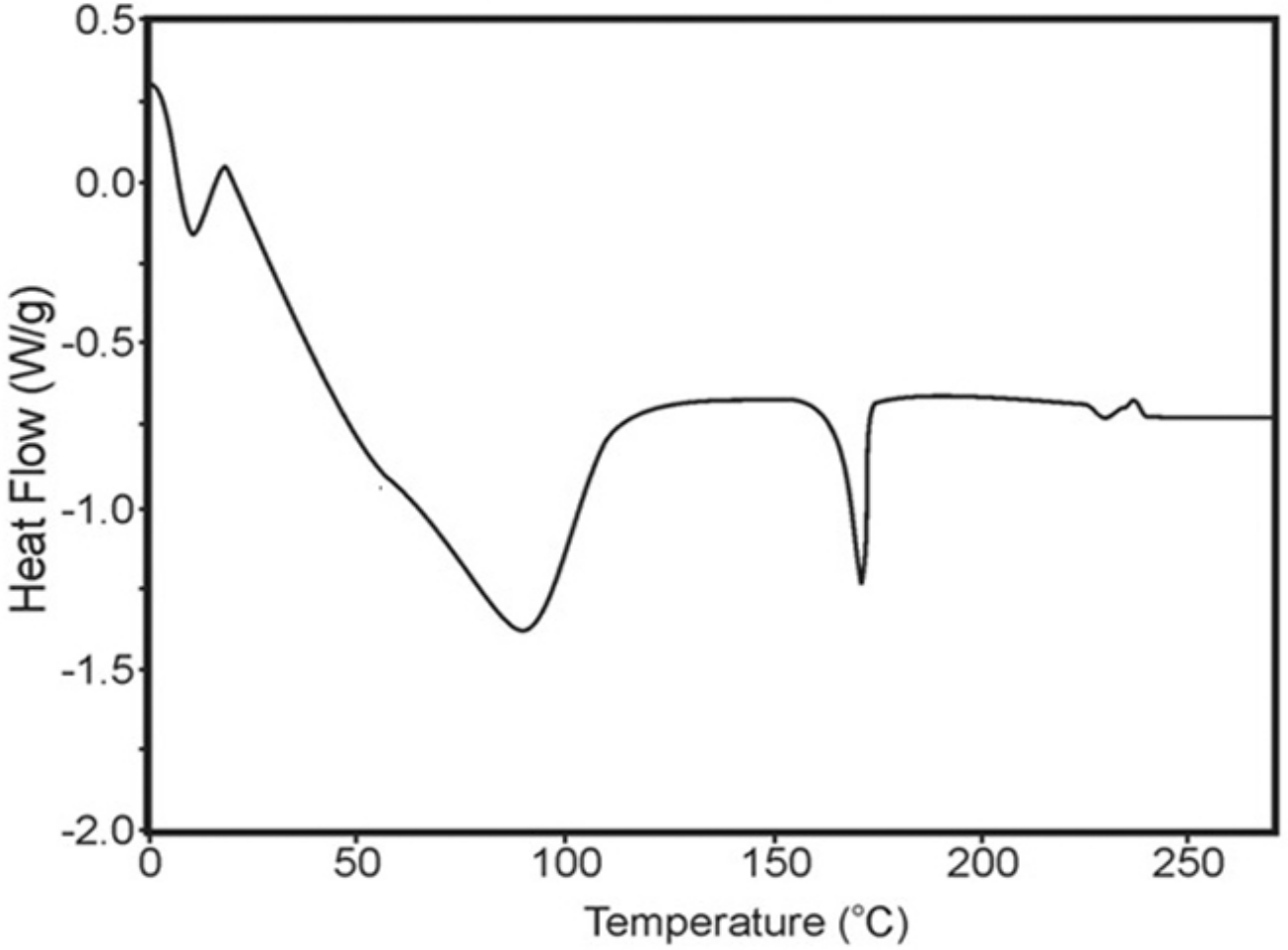


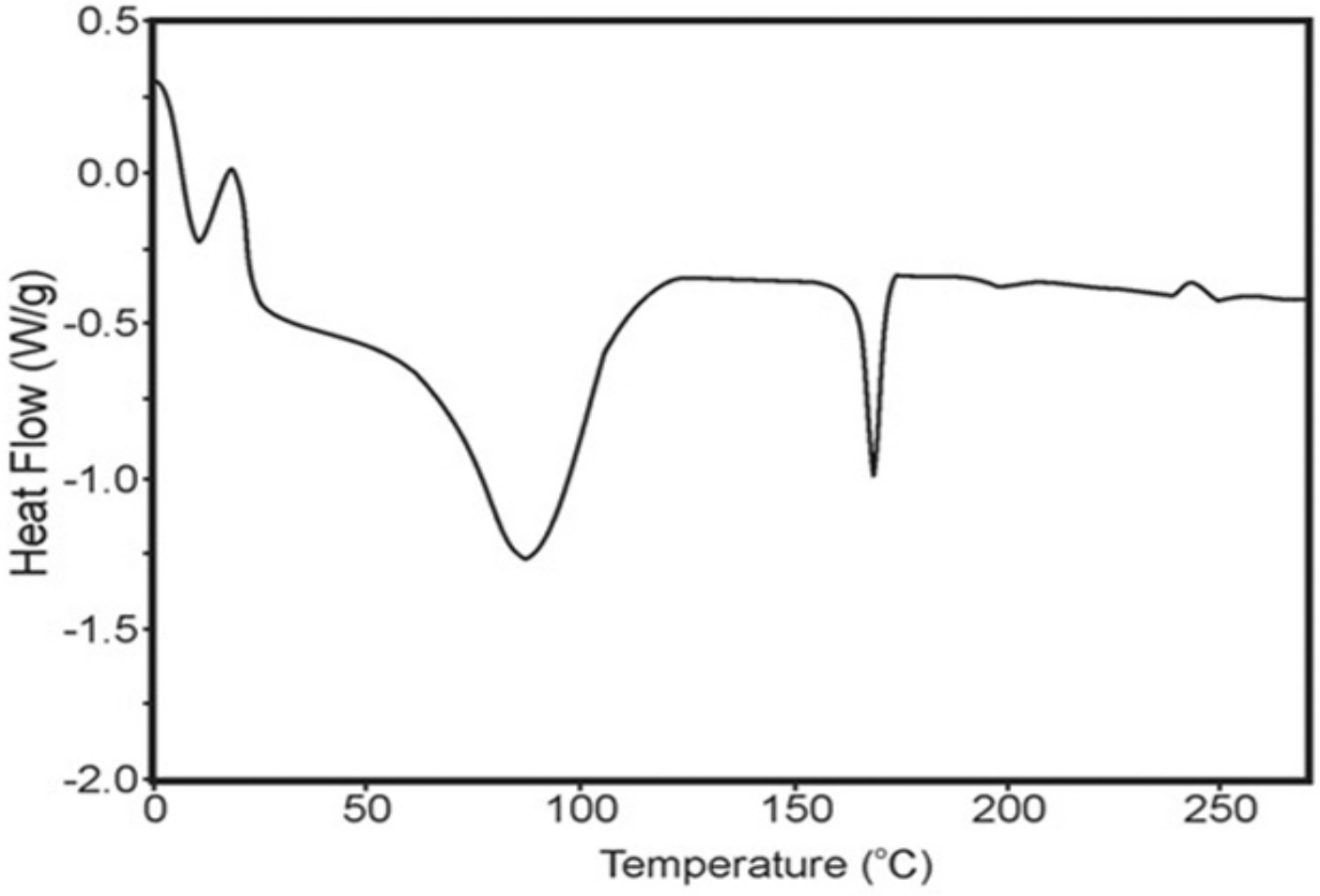
Figure.9 **Pure Itraconazole**



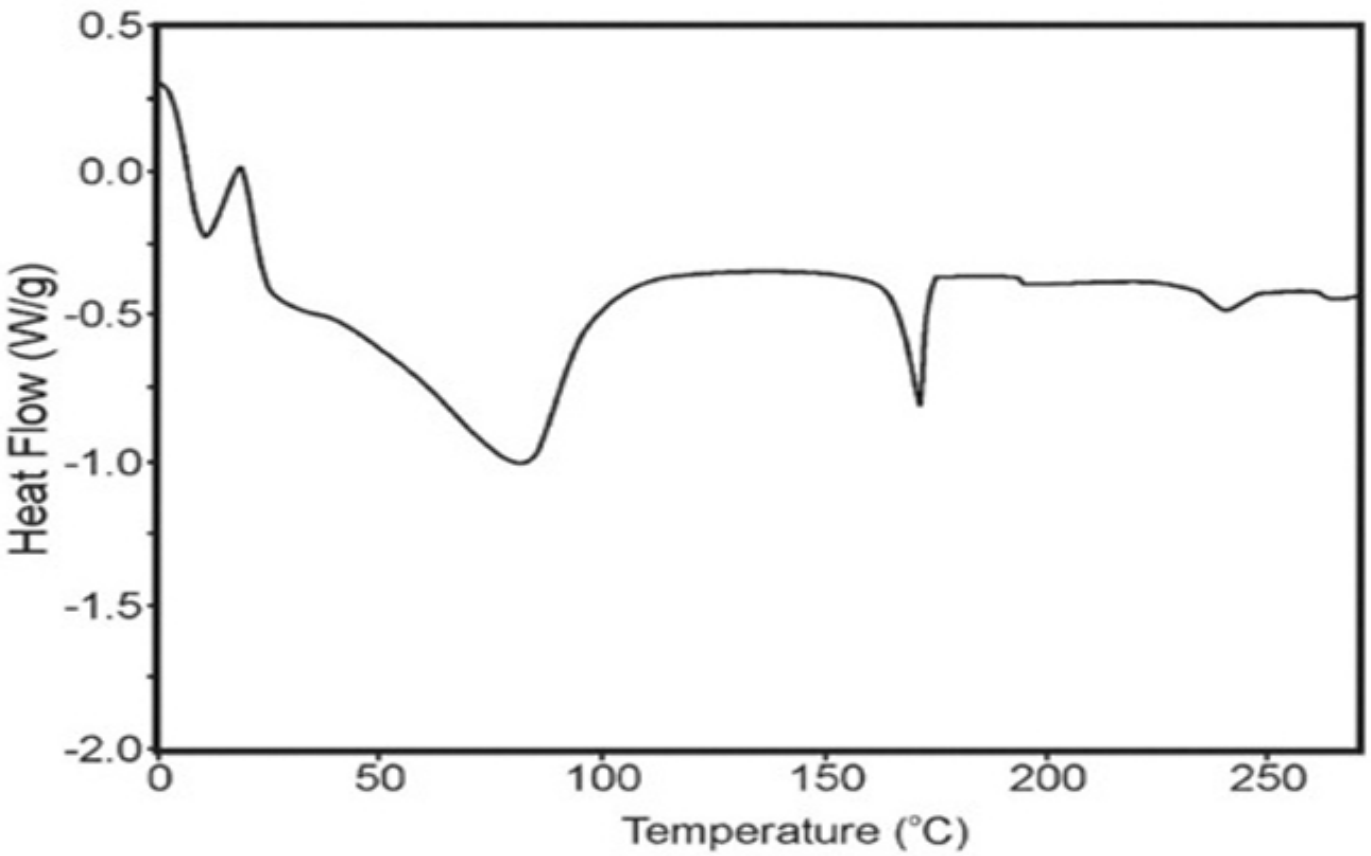
10 Pure β-cyclodextrin



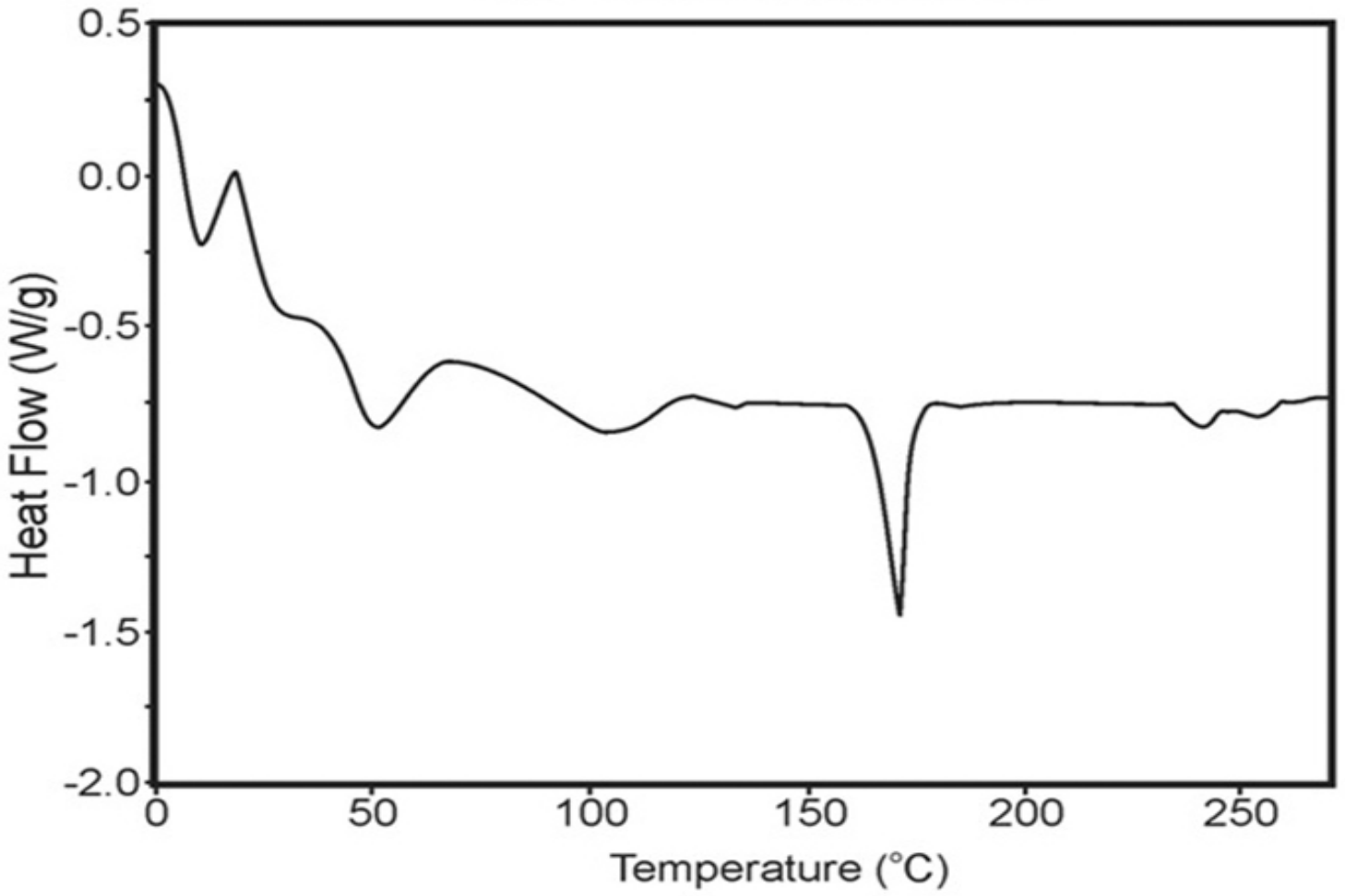
Physical mixture of Itraconazol β-cyclodextrin complex 1:2



Physical mixture of Itraconazole β-cyclodextrin complex 1:4



Co-precipitation of Itraconazole β- cyclodextrin complex 1:2



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Co-precipitation of Itraconazole β- cyclodextrin complex 1:4

8.3 PHASE SOLUBILITY STUDY OF ITRACONAZOLE IN pH (1.2, 2.0, 3.0, 4.0)

Phase Solubility Study

Phase solubility study was carried out in order to ascertain the effect of β- cyclodextrin on the solubility characteristics of ITC. The results are shown in Table 50 and figure 88-91 Solubility of ITC was increased as the concentration of β-cyclodextrin increases in pH 1.2 and in remaining pH it is decreased compare to the pH 1.2.

Table 7: Comparative phase solubility study of Itraconazole(mcg/ml) in different pH buffers

S.no	β–cyclodextrin (µg/ml)	pH 1.2	pH 2.0	pH 3.0	pH 4.0
1	0	1.5±0.3	1.3±0.16	1.2±0.18	1.102±0.132
2	10	2.7±0.12	2.0±0.7	2.4±0.34	2.6±0.164
3	20	3.3±0.6	3.5±0.26	3.2±0.11	3.4±0.173
4	30	5.2±0.51	4.7±0.51	4.4±0.21	4.8±0.185
5	40	8.1±0.23	7.9±0.9	7.6±0.168	7.2±0.561

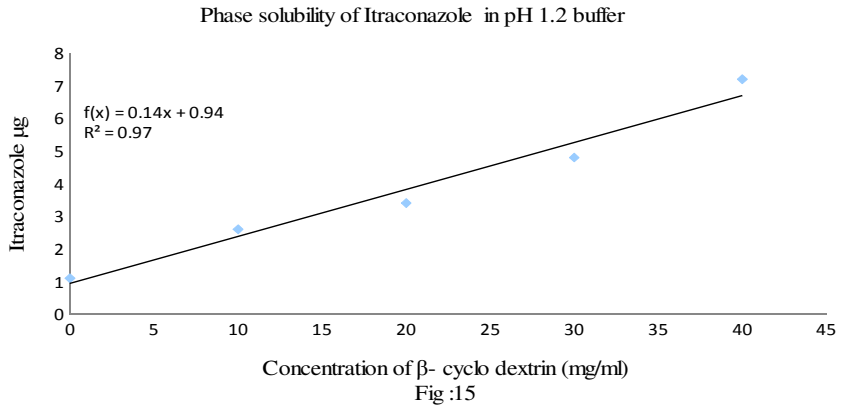

Fig :15

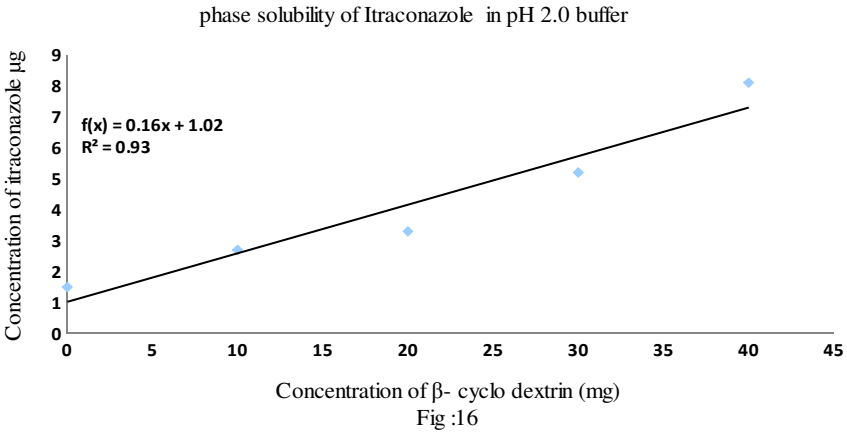

Fig :16

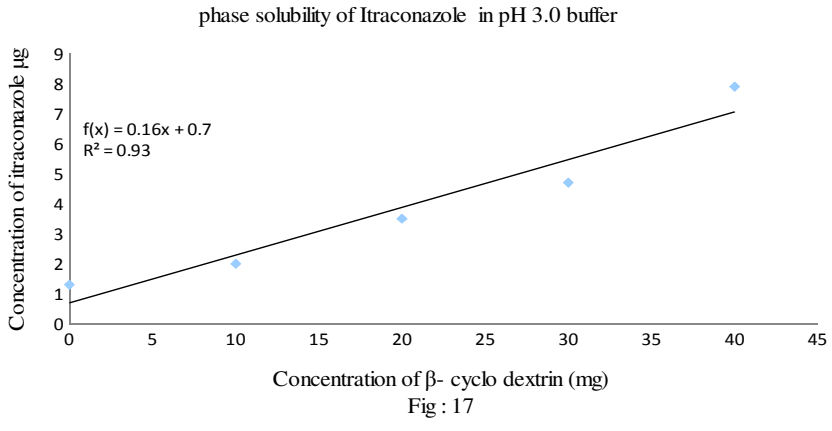

Fig : 17

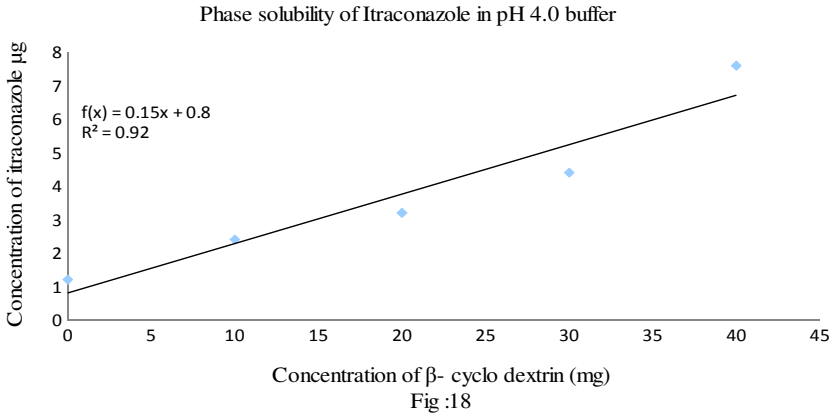

Fig :18

8.4: CALIBRATION CURVES OF ITRACONAZOLE IN DIFFERENT pH (1.2, 2.0, 3.0, 4.0)

Table 8: Calibration curve of Itraconazole in pH 1.2

Concentration (µg/ml)	Absorbance
0	0
2	0.145
4	0.264
6	0.407
8	0.541
10	0.679

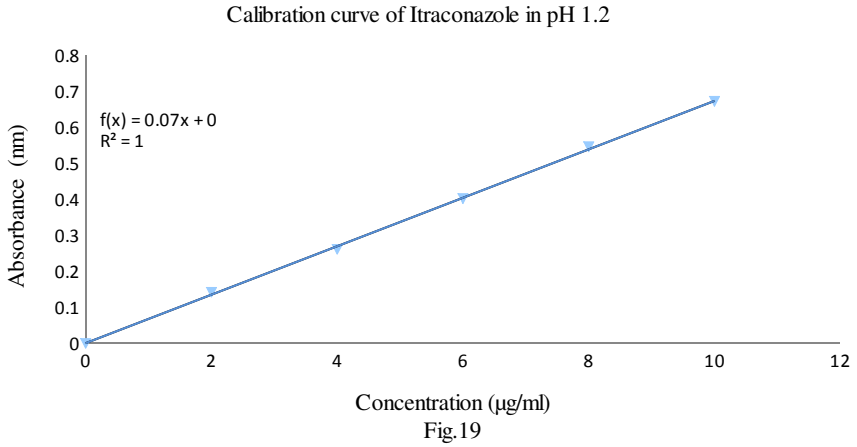

Fig.19

Table 9: Calibration curve of Itraconazole in pH 2.0

Concentration (µg/ml)	Absorbance
0	0
2	0.269
4	0.351
6	0.447
8	0.554
10	0.623

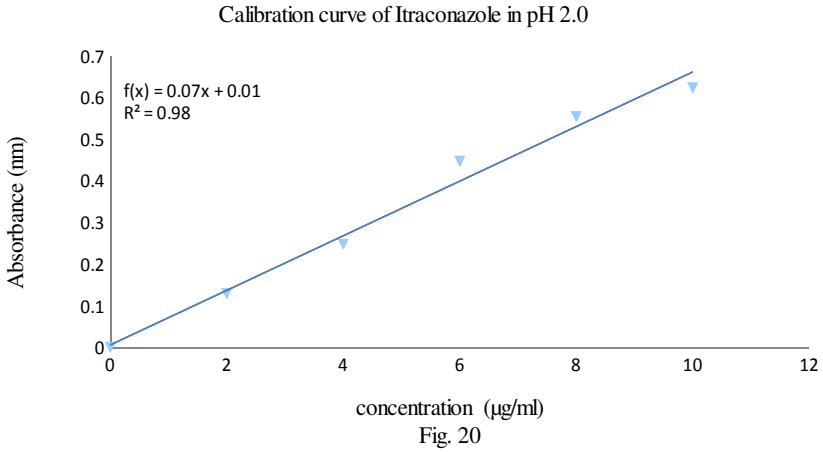

Fig. 20

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Table 10: Calibration curve of Itraconazole in pH 3.0

Concentration (µg/ml)	Absorbance
0	0
2	0.135
4	0.298
6	0.424
8	0.571
10	0.684

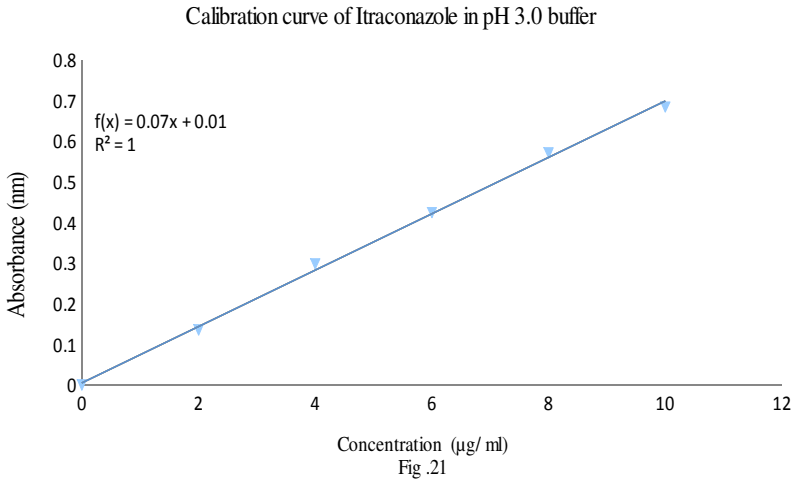


Fig .21

Table 11: Calibration curve of Itraconazole in pH 4.0

Concentration (µg/ml)	Absorbance
0	0
2	0.129
4	0.253
6	0.425
8	0.569
10	0.634

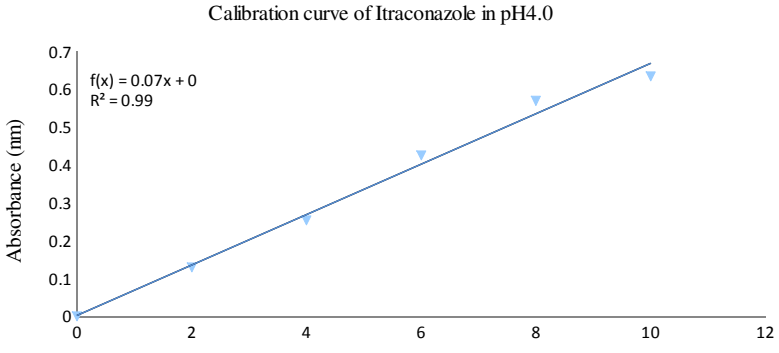


Fig .22

Table 12: *In-vitro* drug release pure Itraconazole in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release*
5	13.4	12.8	12.6	12.93±0.41
10	14.8	16.3	15.7	15.63±0.75
20	22.8	21.9	23.5	22.733±0.8
30	25.6	27.0	25.5	26.03±0.83
45	31.5	32.4	30.8	31.563±0.8
60	34.5	36.1	34.6	35.063±0.89

In-vitro drug release of Itraconazole in pH 1.2

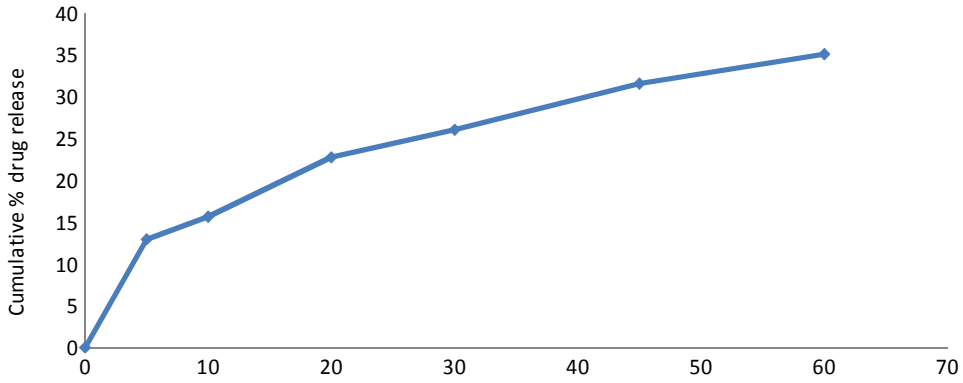


Fig .23

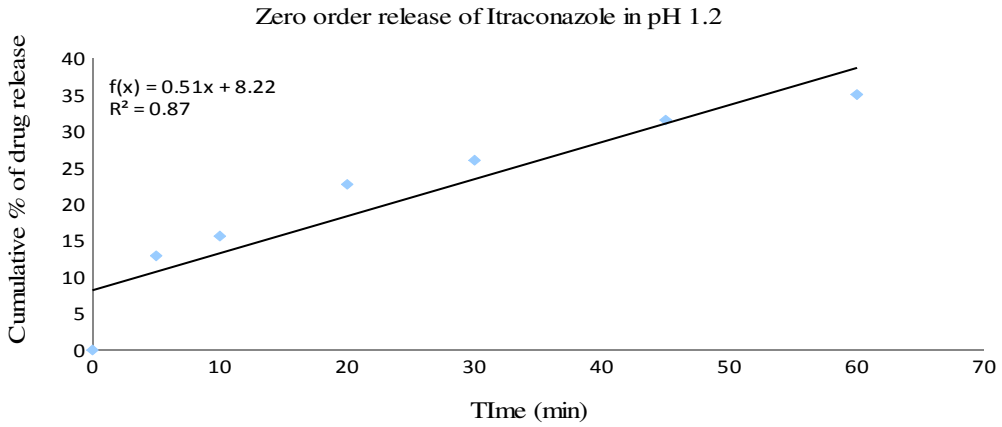


Fig. 24

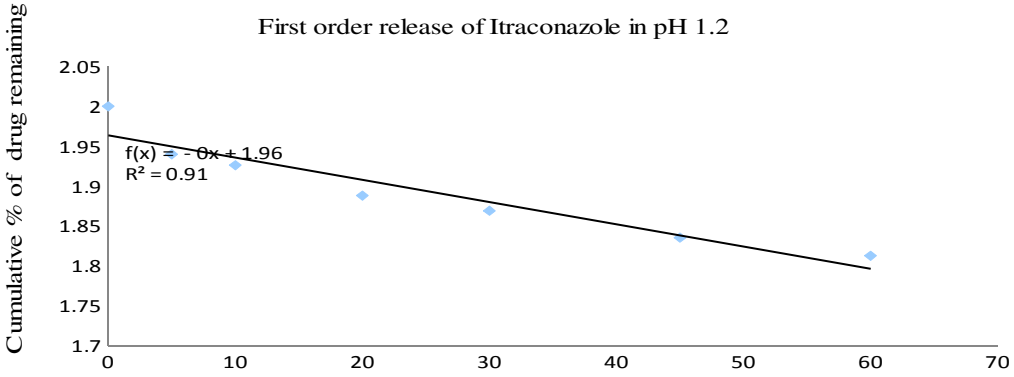


Fig . 25

Table 13: *In-vitro* drug release of physical mixture 1:2 in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	27.4	25.4	26.1	26.4±0.81
10	30.2	38.5	29.7	29.48±0.87
20	31.8	30.7	32.4	31.6±0.86
30	37.4	35.6	36.0	36.33±0.94
45	47.2	48.1	46.8	47.36±0.66

60	57.2	56.8	58.0	57.3±0.61

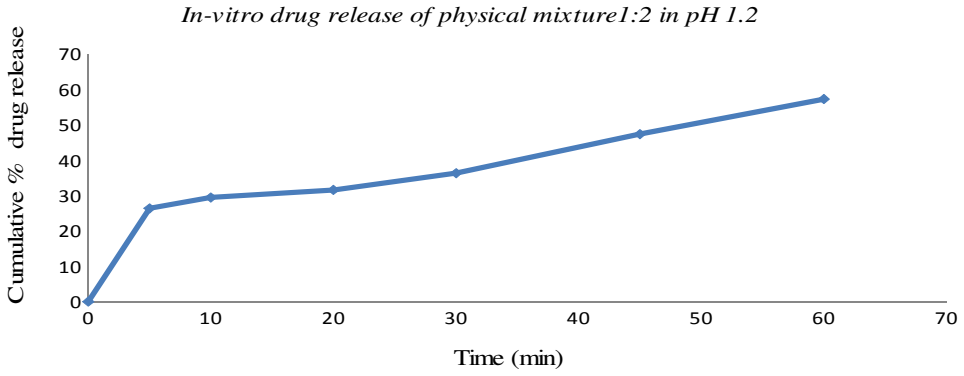


Fig . 26

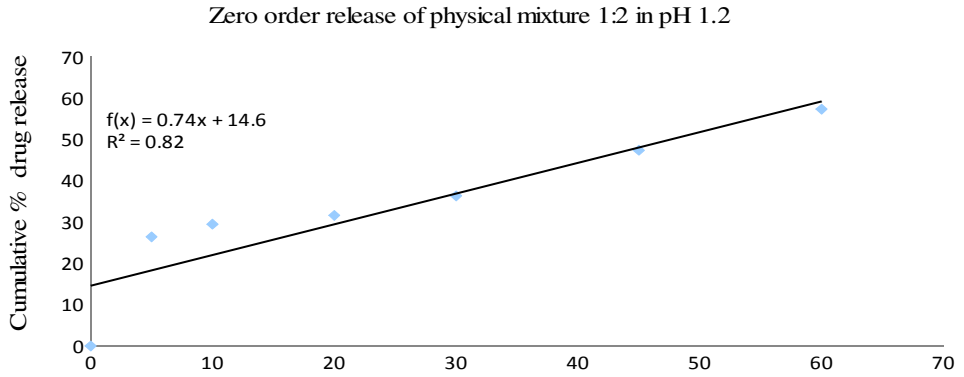


Fig . 27

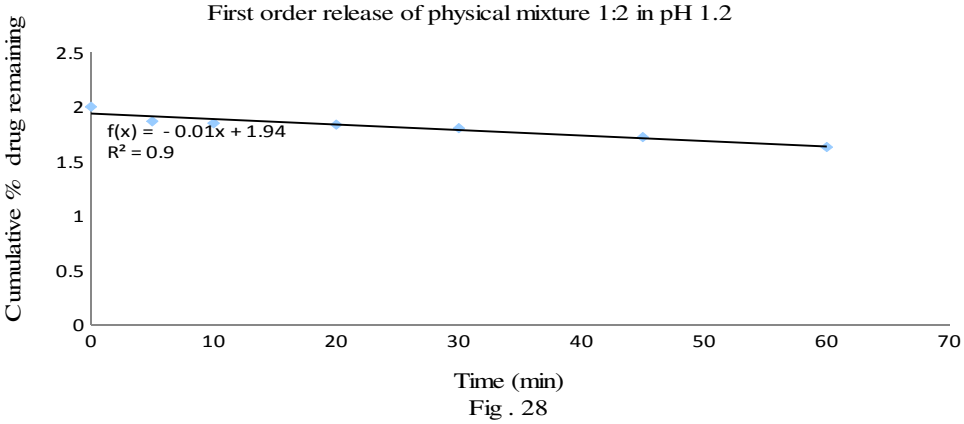


Fig . 28

Table 14: *In-vitro* drug release of co-precipitation 1:2 in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	24.5	30.65	34.2	32.2±1.8
10	37.2	40.7	38.5	38.8±1.7
20	45.0	47.8	42.9	45.2±2.4
30	56.4	52.9	57.3	56.1±1.2
45	68.9	65.7	63.5	67.3±1.6
60	73.5	70.4	75.8	73.2±2.7

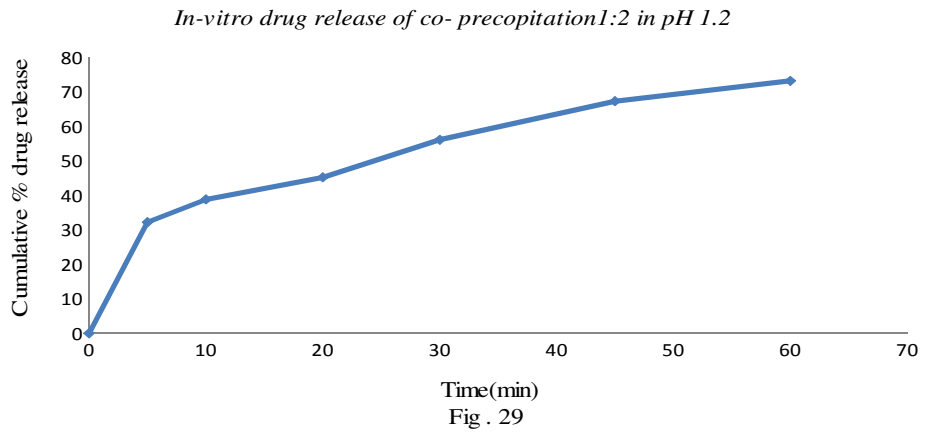


Fig . 29

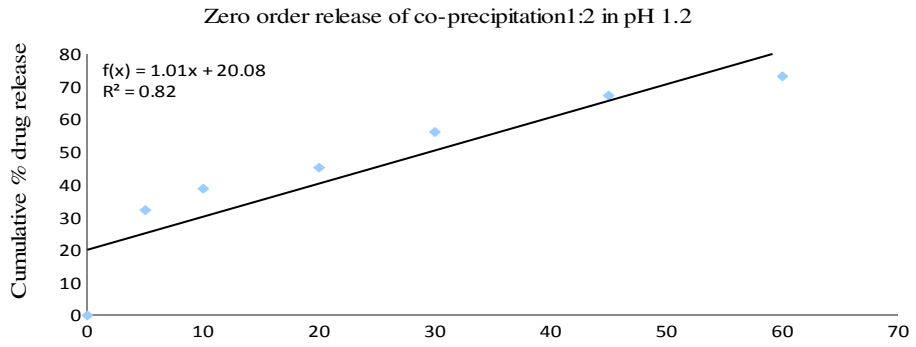


Fig . 30

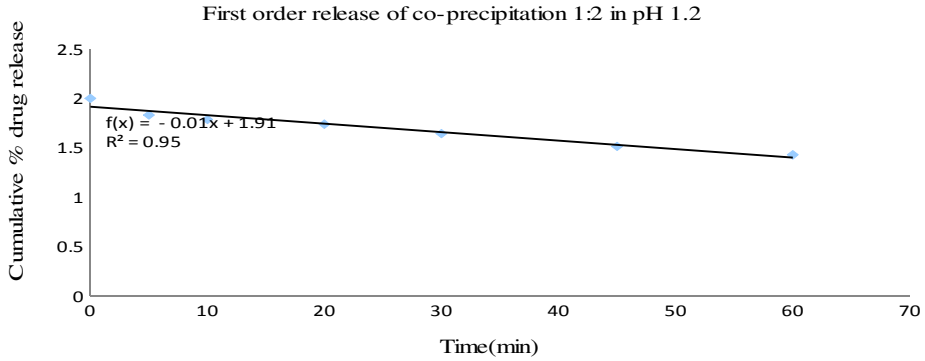


Fig . 31

Table 15: *In-vitro* drug release of physical mixture 1:4 in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	24.4	26.4	23.7	24.8±1.4
10	33.2	32.7	35.08	33.6±1.2
20	39.6	43.4	41.7	41.5±1.9
30	55.3	53.6	56.3	55.0±1.3
45	59.3	57.3	60.4	59.0±1.5
60	66.8	68.2	65.8	66.9±1.2

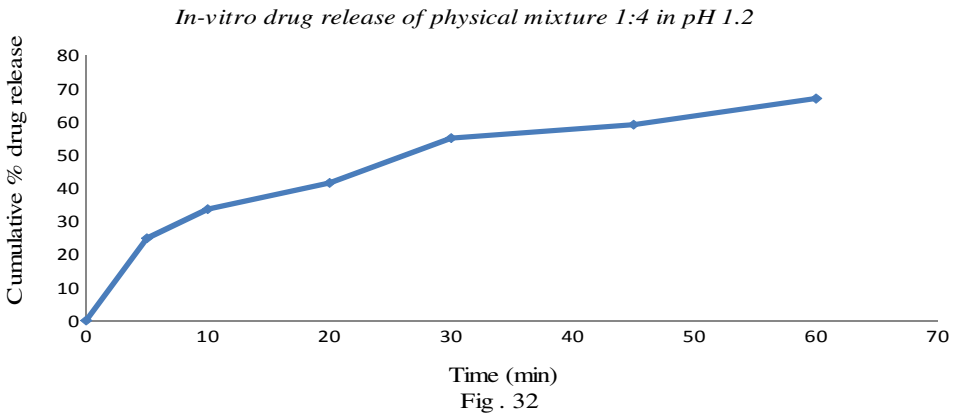


Fig . 32

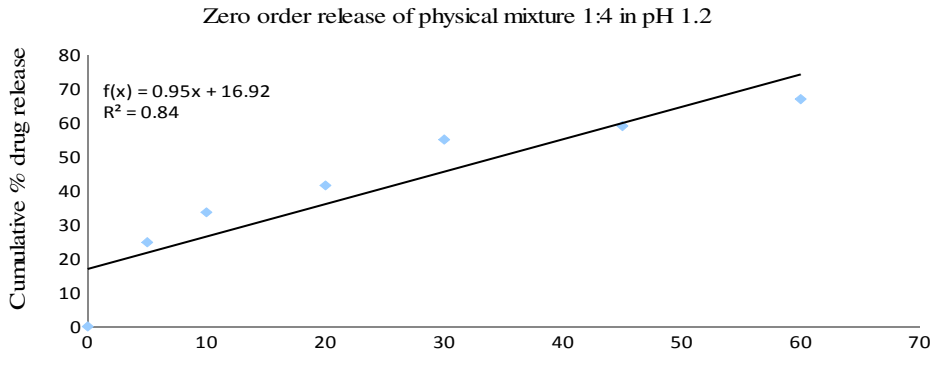


Fig . 33

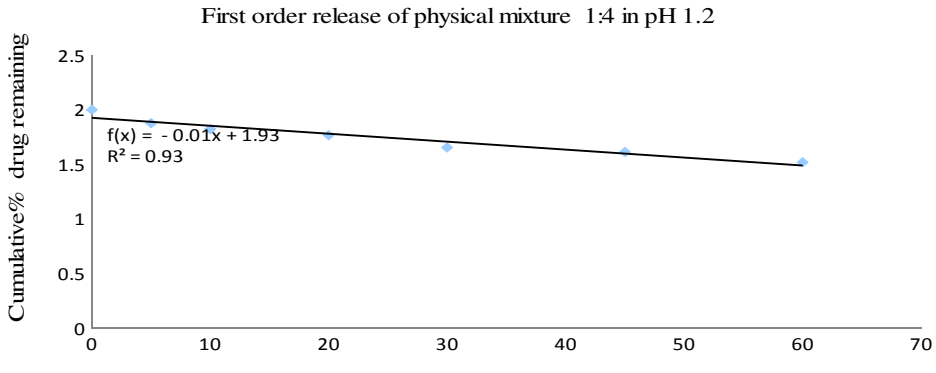


Fig . 34

Table 16: *In-vitro* drug release of co-precipitate 1:4 in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	29.8	28.7	31.8	30.1±1.57
10	36.9	35.7	39.6	37.4±1.99
20	49.9	50.4	52.5	50.93±1.38
30	63.6	62.4	65.7	63.9±1.6
45	67.8	68.1	71.4	69.1±1.99
60	82.5	81.1	84.7	82.7±1.8

In-vitro drug release of co-precipitate1:4 in pH 1.2

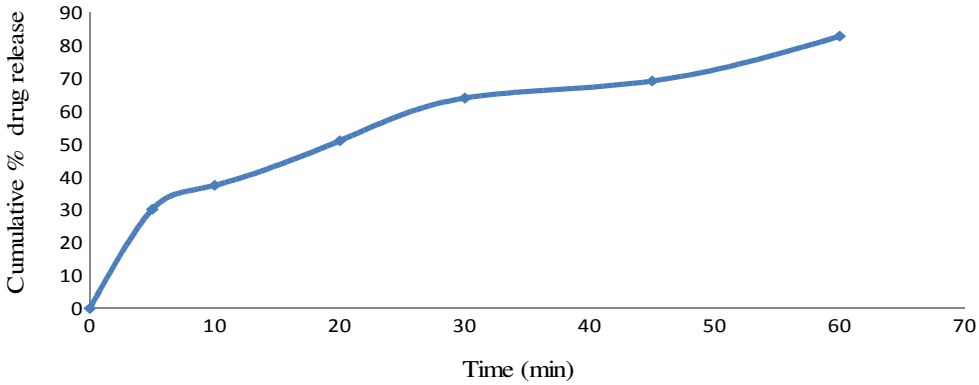


Fig . 35

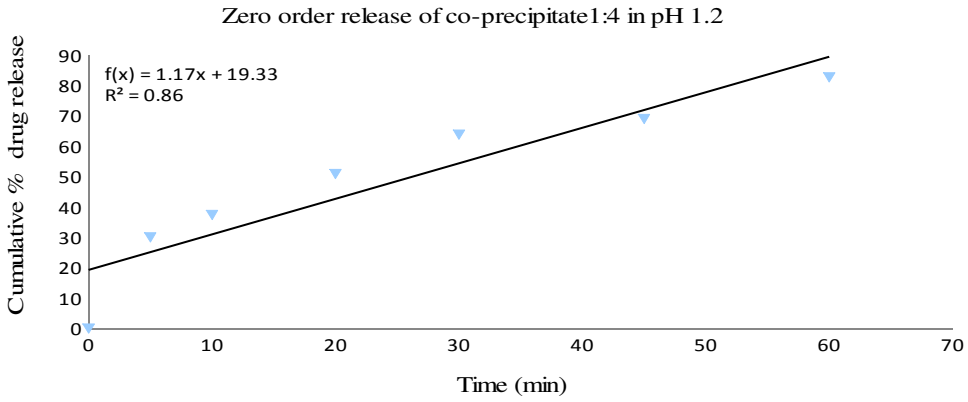


Fig. 36

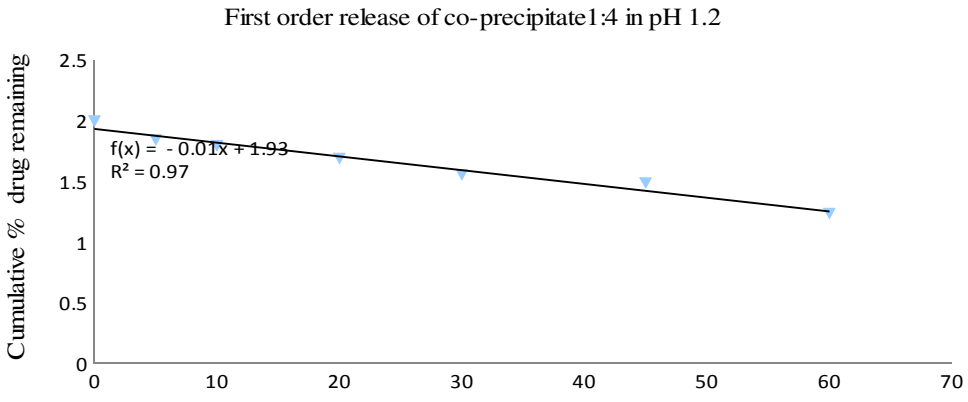


Fig. 37

Table 17: *In-vitro* drug release of Itraconazole in pH 2.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	12.6	13.8	15.3	13.9±1.3
10	23.5	21.4	20.6	21.8±1.4
20	28.8	26.2	27.7	27.5±1.3
30	35.7	36.8	34.5	35.6±1.1
45	39.4	37.4	40.6	39.1±1.6

60	43.7	41.3	42.1	42.3±1.2

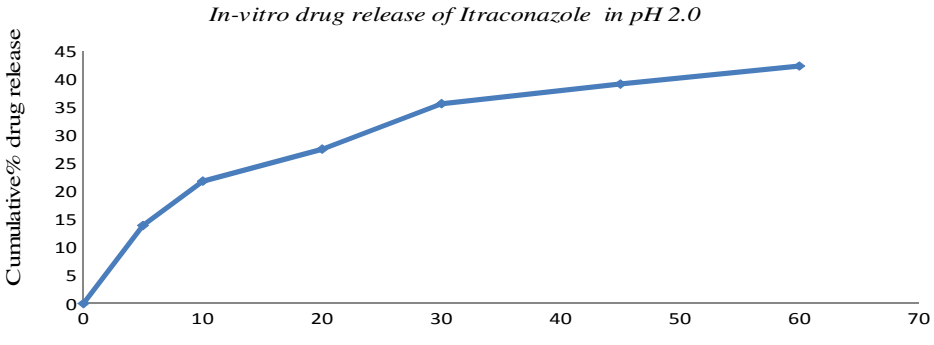


Fig . 38

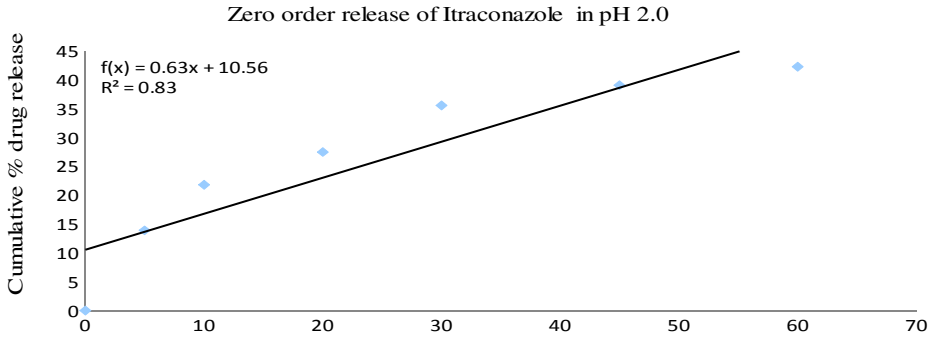


Fig . 39

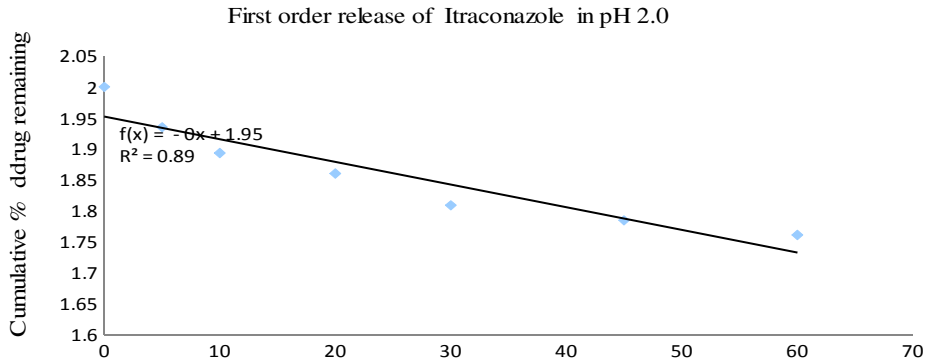


Fig . 40

Table 18: *In-vitro* drug release of physical mixture1:2 in pH 2.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	23.6	21.4	24.7	23.2±1.6
10	28.8	27.9	30.1	28.9±1.1
20	36.9	34.8	38.5	36.7±1.8
30	46.5	43.7	45.4	45.2±1.4
45	58.4	54.5	56.3	56.4±1.9
60	62.9	57.6	58.5	59.6±2.8

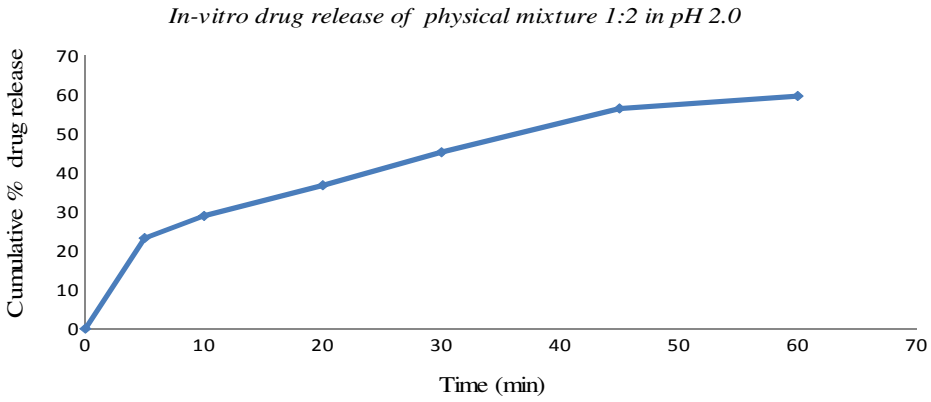


Fig . 41

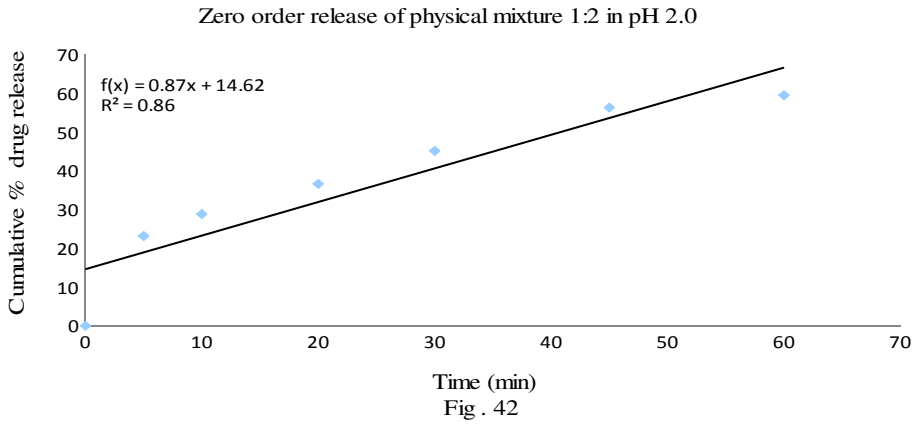


Fig . 42

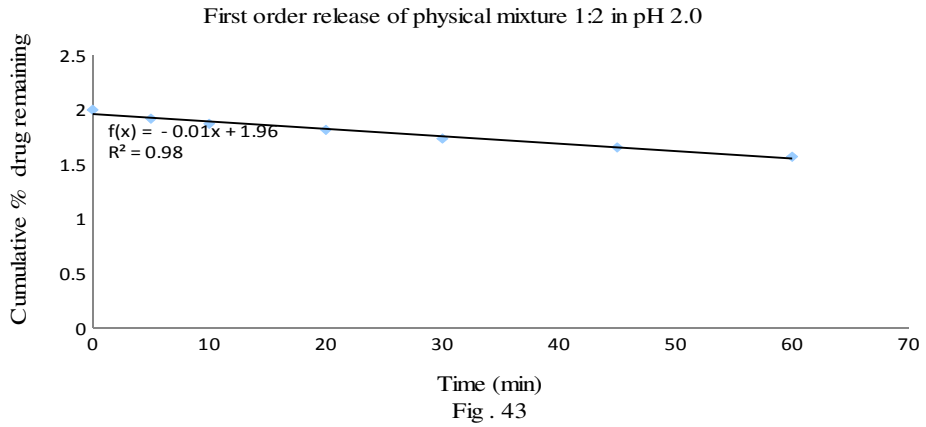


Fig . 43

Table 19: *In-vitro* drug release of co-precipitation 1:2 in pH 2.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	22.5	24.6	25.4	24.16±1.49
10	34.9	36.7	38.2	36.6±1.6
20	45.4	44.3	46.9	45.53±1.30
30	62.7	59.3	60.6	60.86±1.71
45	73.6	71.3	72.3	72.4±1.15
60	78.8	79.2	82.4	81.1±1.9

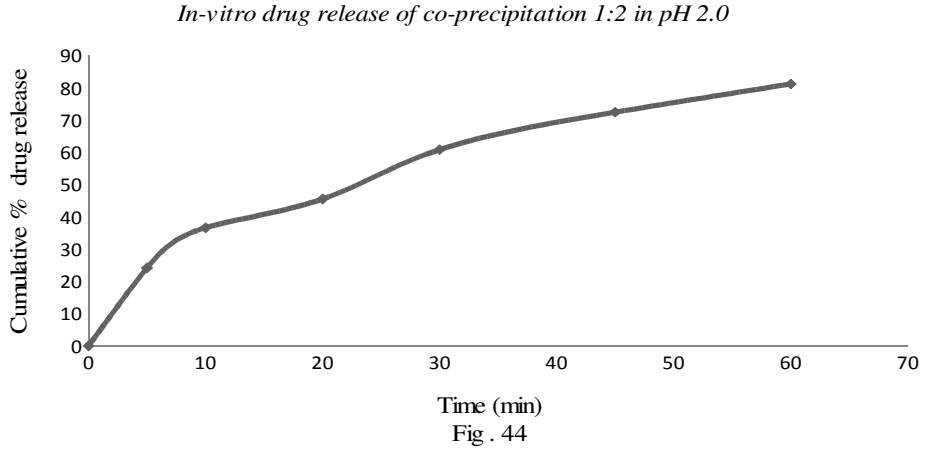


Fig . 44

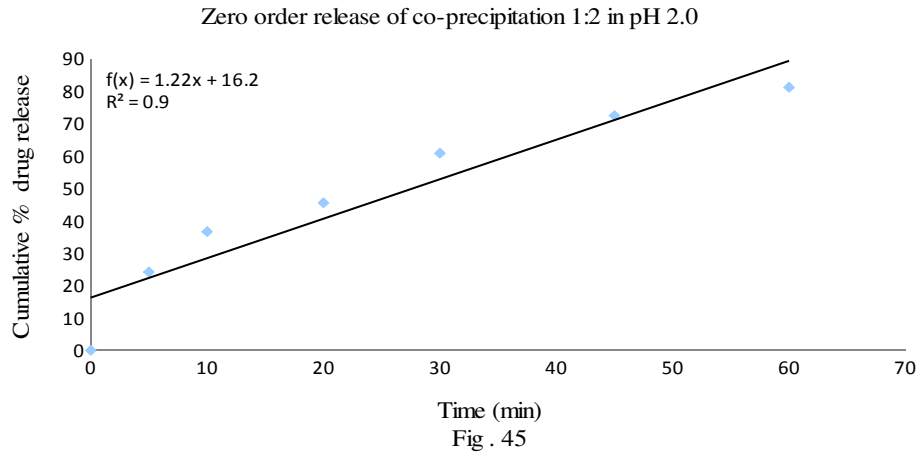


Fig . 45

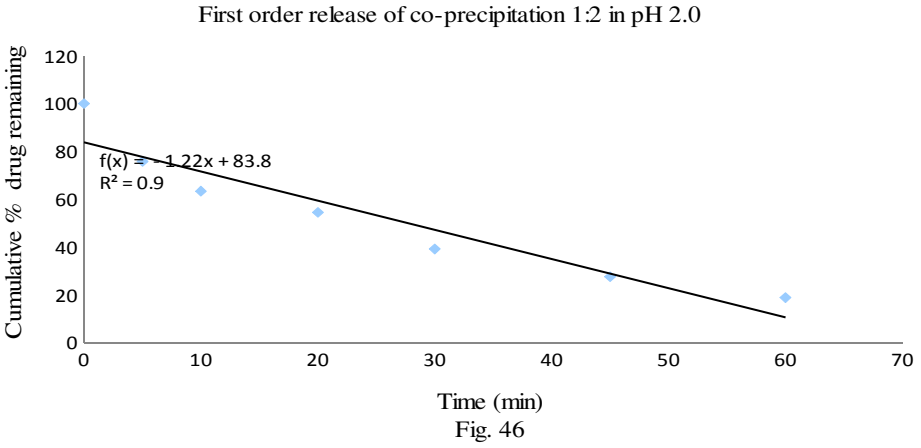


Fig. 46

Table 20: *In-vitro* drug release of physical mixture 1:4 in pH 2.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	21.9	20.6	22.9	21.8± 1.15
10	27.9	26.6	28.5	27.6±0.97
20	35.4	37.8	38.6	37.2±1.63
30	57.4	56.9	59.3	57.8±1.26

45	62.7	61.9	64.8	63.13±1.49
60	68.5	67.5	69.5	68.5±1.0

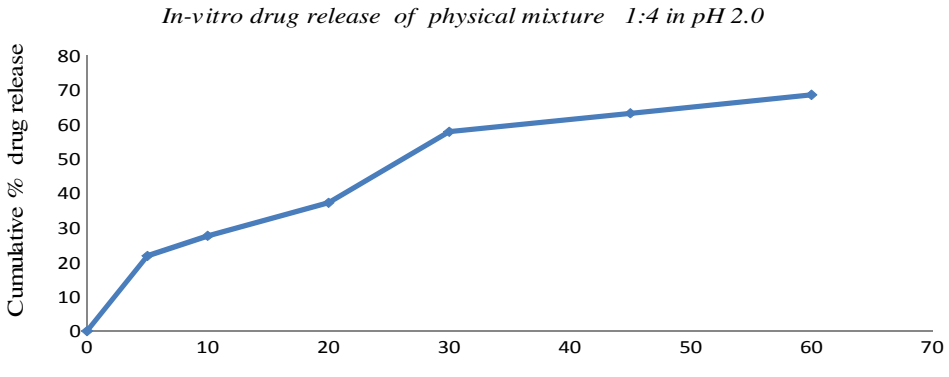


Fig . 47

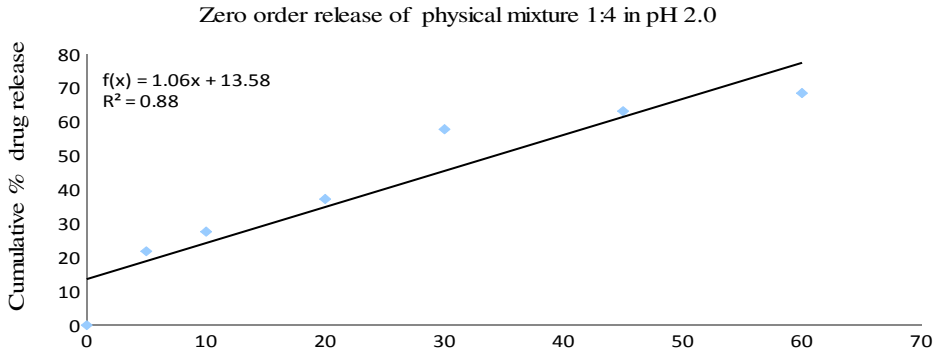


Fig . 48

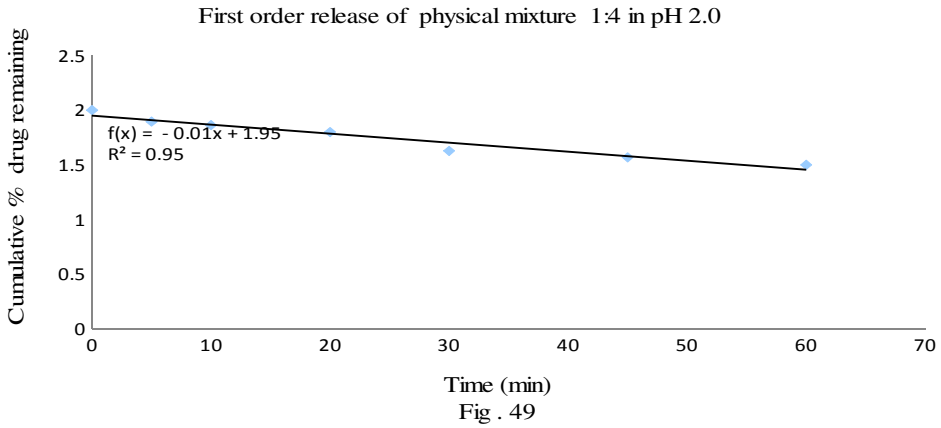


Fig . 49

Table 21: *In-vitro* drug release of co-precipitation 1:4 in pH 2.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	37.1	35.3	38.5	36.9±1.6
10	45.9	47.2	48.7	47.2±1.4
20	58.4	56.9	53.6	56.3±2.4
30	69.2	65.5	67.4	67.3±2.4
45	74.3	76.9	78.3	76.5±2.03
60	83.8	84.3	81.5	83.2±1.49

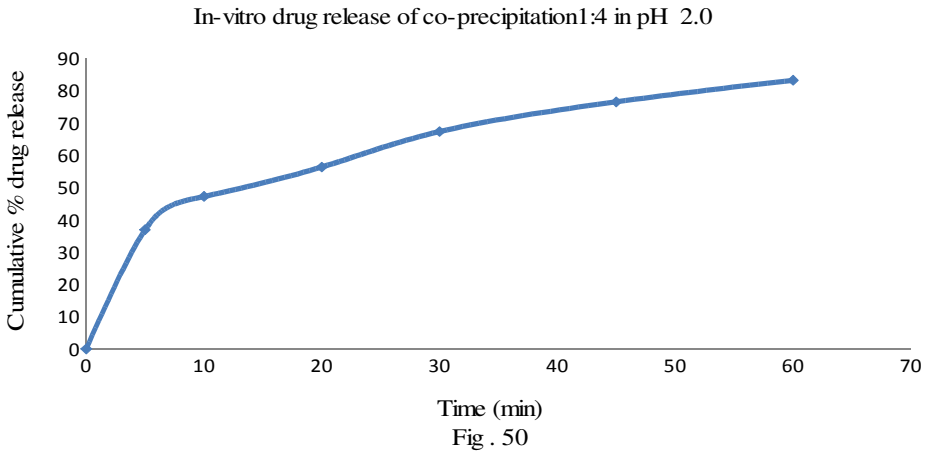


Fig . 50

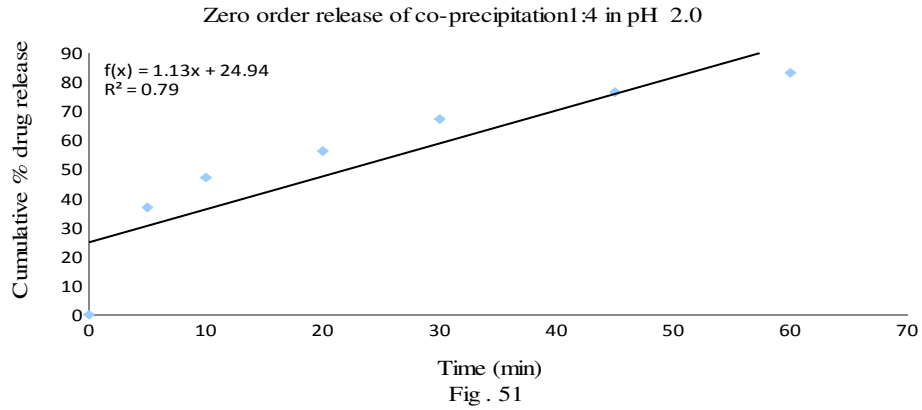


Fig . 51

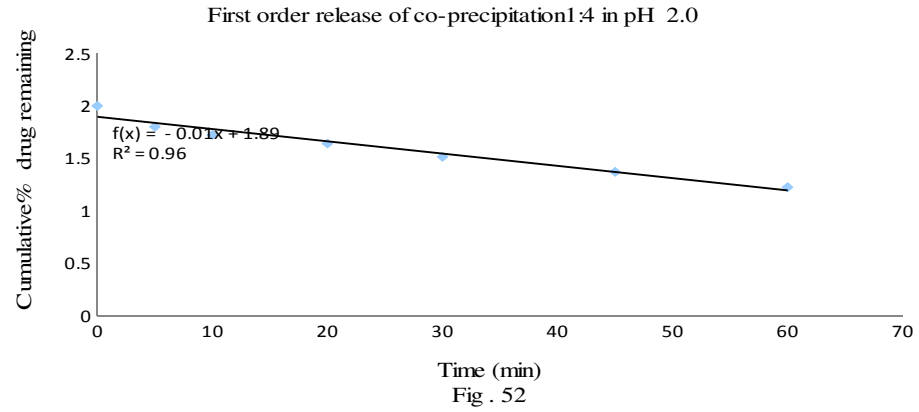


Fig . 52

Table 22*: In-vitro* drug release of Itraconazole in pH 3.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	18.6	15.8	19.7	18.0±2.01
10	22.6	20.7	24.3	22.5±1.8
20	32.3	35.1	33.6	33.6±1.4
30	37.8	36.4	38.2	37.4±0.9
45	43.6	41.9	44.6	43.3±1.3
60	44.5	46.7	48.1	46.43±1.8

In-vitro drug release of Itraconazole in pH 3.0

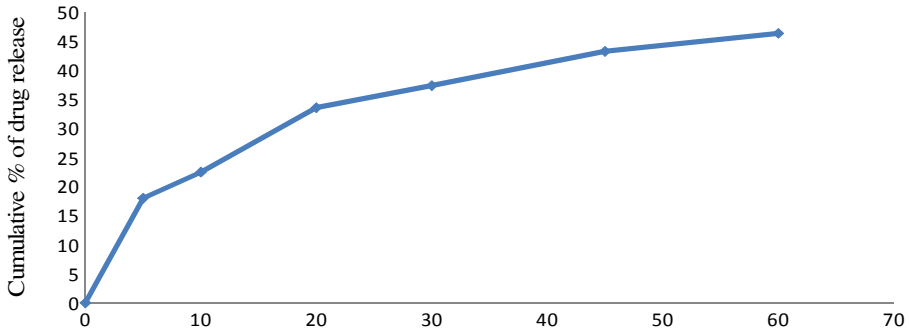


Fig . 53

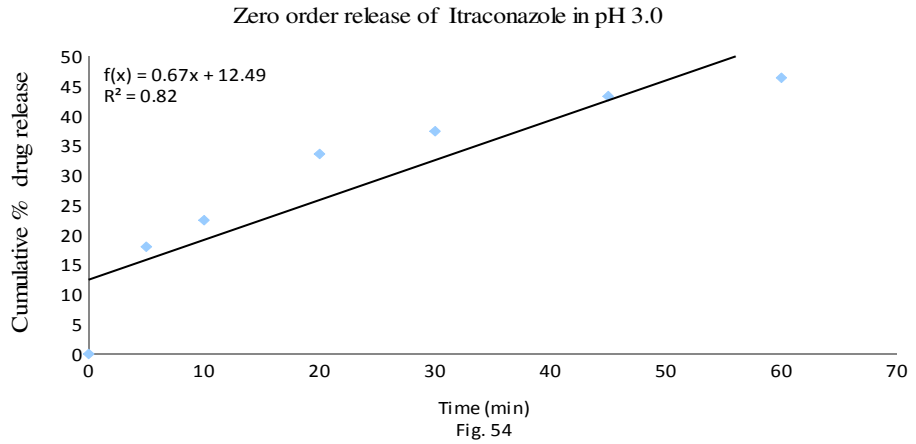


Fig. 54

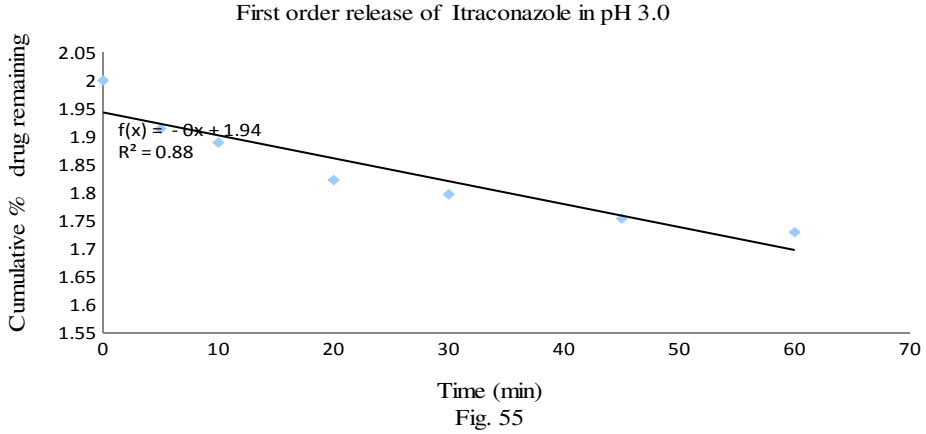


Fig. 55

Table 23: *In-vitro* drug release of physical mixture 1:2 in pH 3.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	15.3	18.9	16.8	17.0±1.8
10	26.9	25.2	24.6	25.5±1.1
20	35.4	36.0	34.9	34.4±o.5
30	47.7	45.5	46.7	46.6±1.2
45	53.5	56.2	54.8	54.8±1.3

60	64.2	62.4	62.3	62.9±1.0

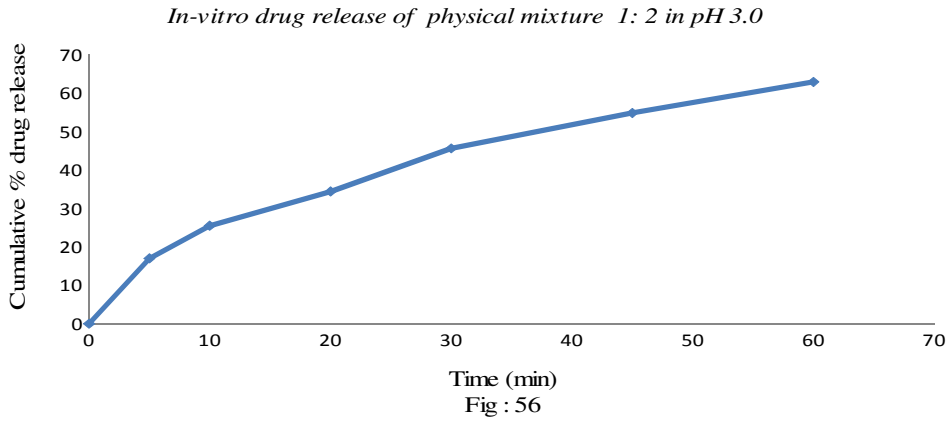


Fig : 56

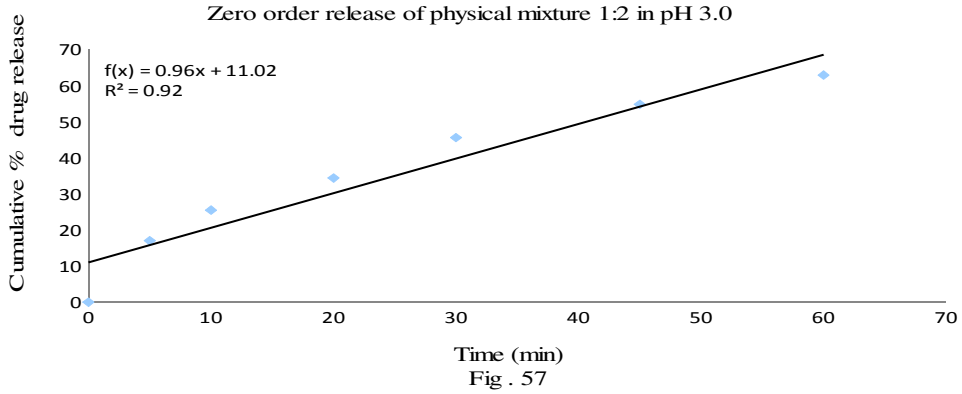


Fig . 57

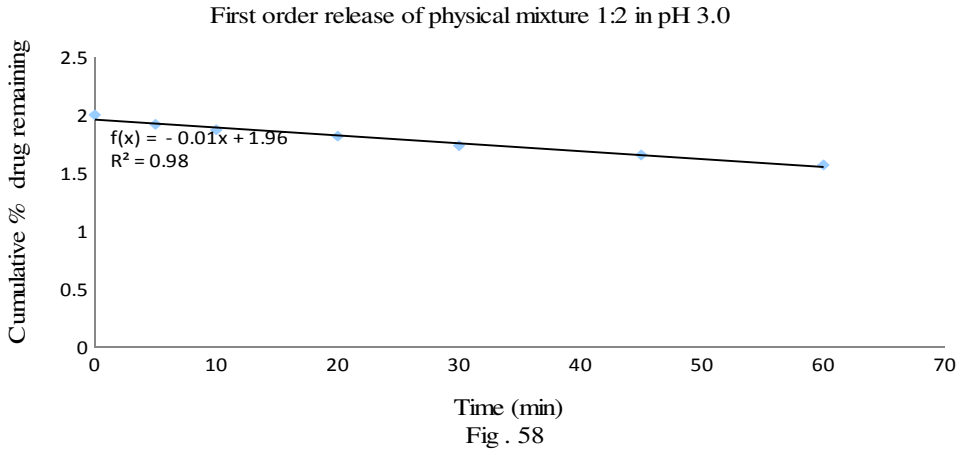


Fig . 58

Table 24: *In-vitro* drug release of Co- precipitation 1:2 in pH 3.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	30.6	32.4	31.6	31.5 ±0.9
10	36.2	37.8	36.9	36.9 ±0.8
20	44.5	43.2	42.8	43.5 ±0.85
30	51.9	50.7	52.4	51.6 ±0.87
45	68.4	69.4	67.5	68.4 ±1.0
60	79.3	78.8	77.9	78.5 ±0.72

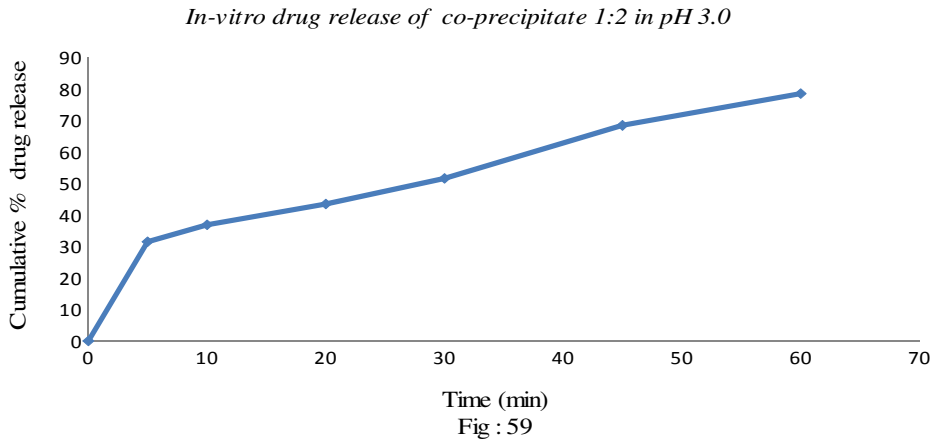


Fig : 59

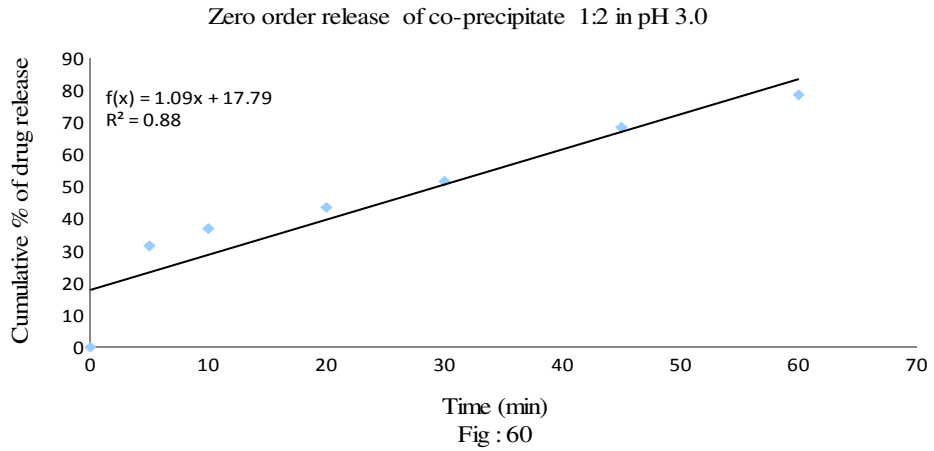


Fig : 60

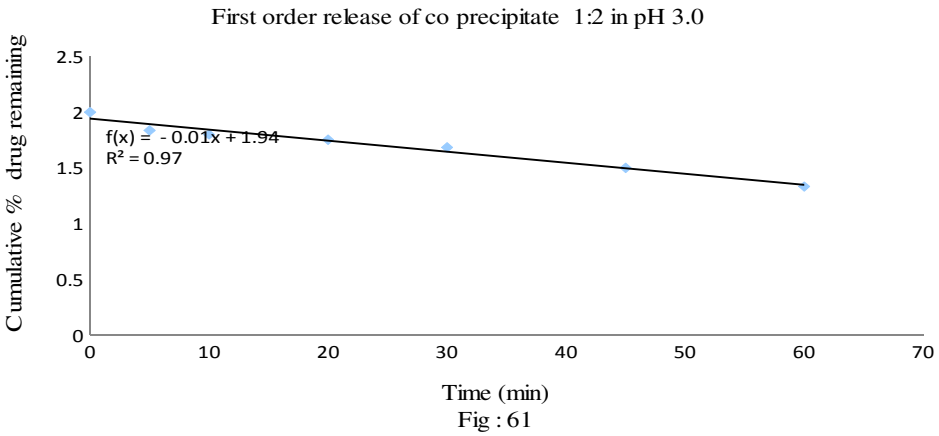


Fig : 61

Table 25: *In-vitro* drug release of physical mixture 1:4 in pH 3.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	30.1	28.6	29.7	29.46±0.77
10	34.2	32.4	33.8	33.4±0.94
20	41.6	43.1	42.4	42,3±0.75
30	51.7	52.1	53.5	52.34±0.94
45	63.4	64.7	62.8	63.43±0.97
60	69.8	71.4	70.3	70.5±0.81

In vitro drug release of physical mixture 1:4 in pH 3.0

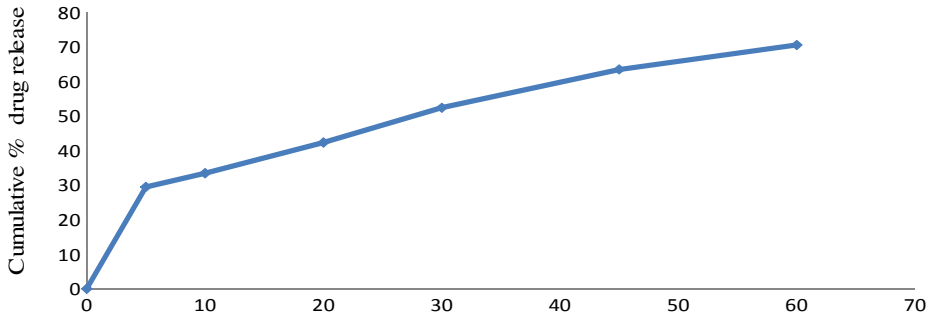


Fig : 62

Zero order release of physical mixture 1:4 in pH 3.0

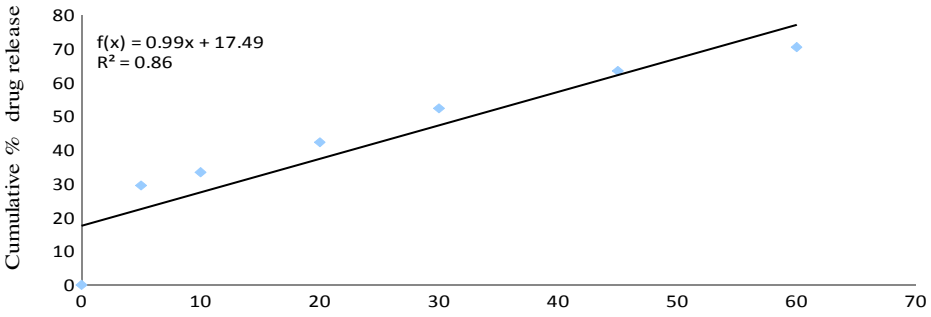


Fig : 63

First order release of physical mixture 1:4 in pH 3.0

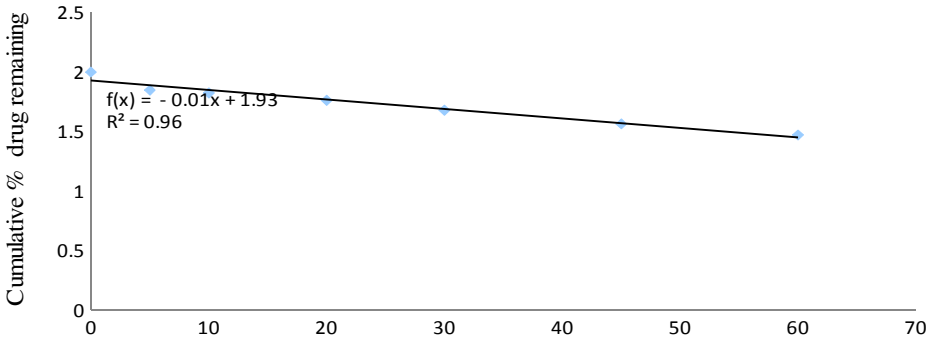


Fig :64

Table 26: *In-vitro* drug release of co-precipitation 1:4 in pH 3.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	37.1	35.3	38.5	36.9±1.6
10	45.9	47.2	48.7	47.2±1.4
20	58.4	56.9	53.6	56.3±2.4
30	69.2	65.5	67.4	67.3±2.4
45	74.3	76.9	78.3	76.5±2.03
60	83.8	84.3	81.5	83.2±1.49

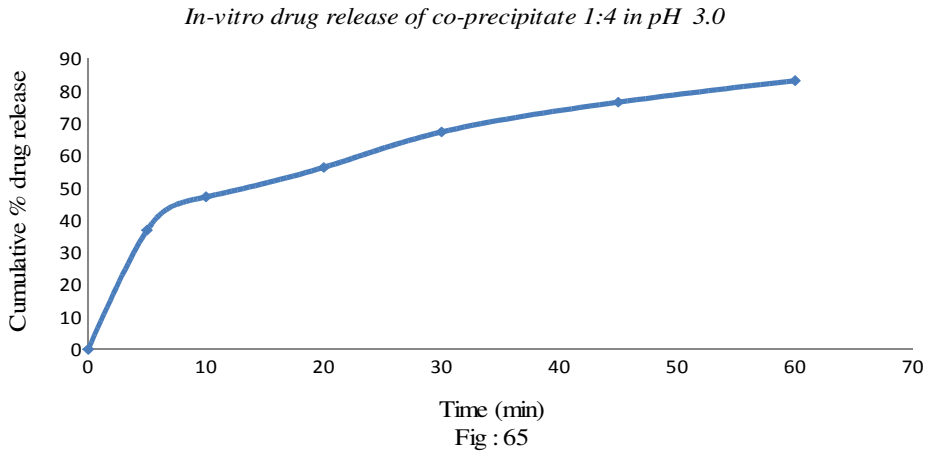


Fig : 65

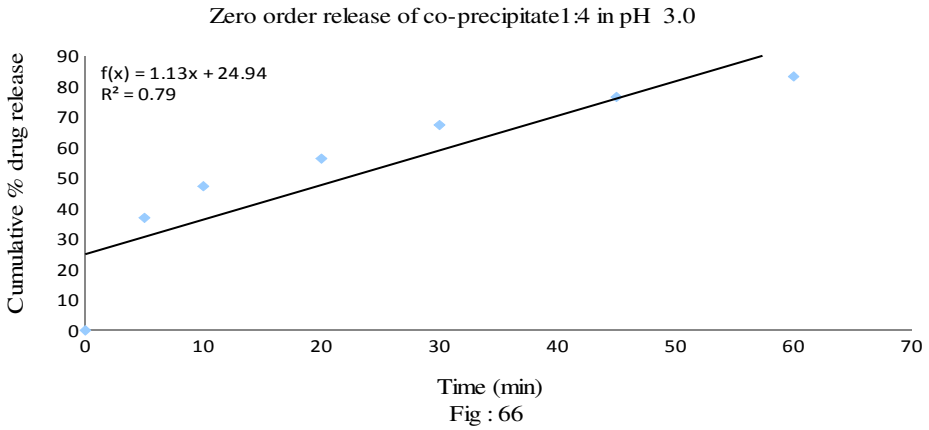


Fig : 66

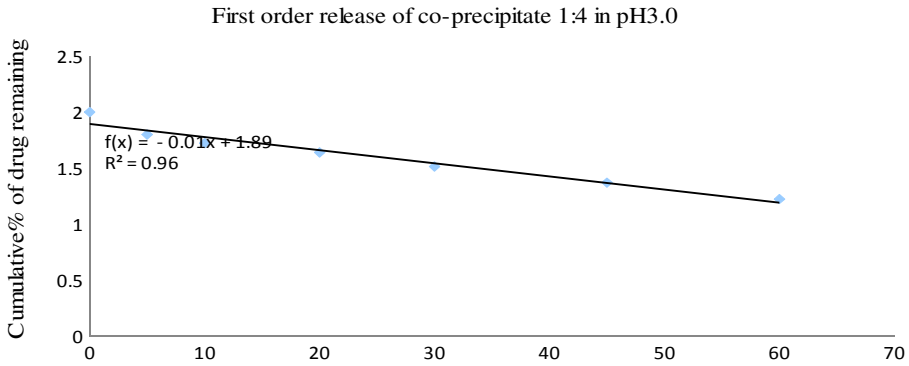


Fig : 67

Table 27: *In-vitro* drug release of Itraconazole in pH 4.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	19.4	23.1	21.9	21.46±1.8
10	28.5	31.6	29.0	29.7±1.6
20	37.4	39.8	35.9	37.7±1.9
30	47.3	44.7	46.6	46.2±1.3
45	51.6	53.6	52.1	52.4±1.04
60	59.4	62.4	60.81	60.8±1.5

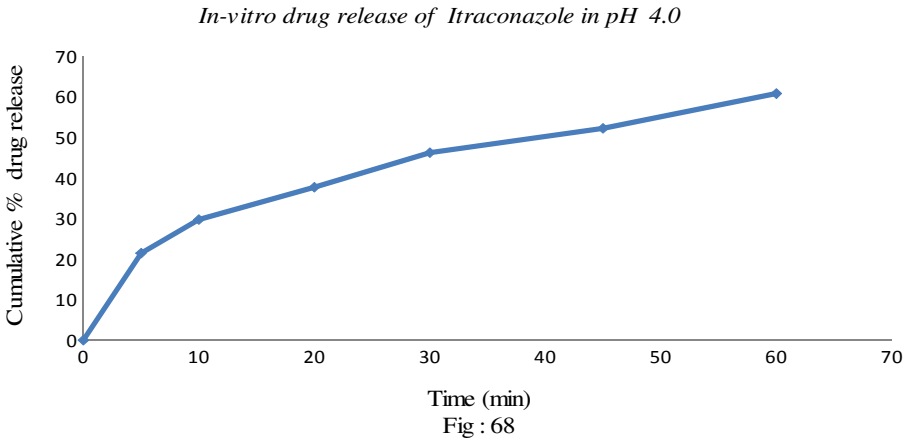


Fig : 68

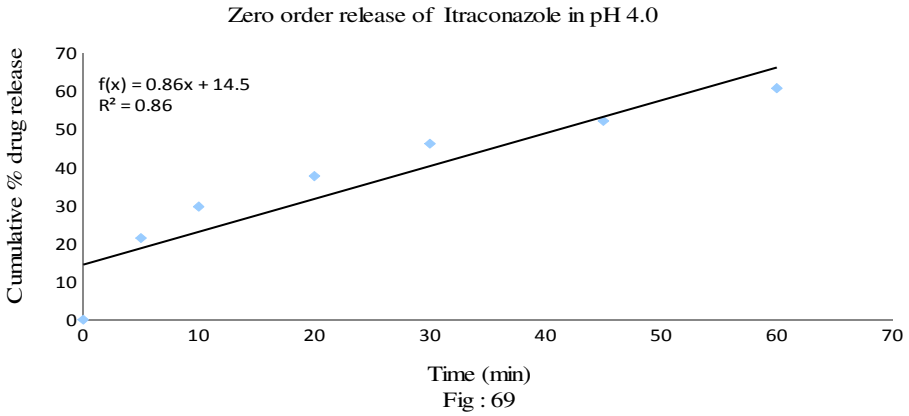


Fig : 69

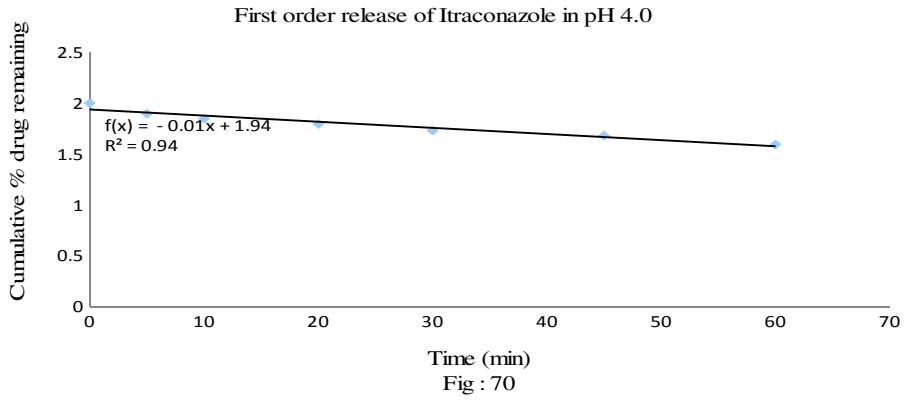


Fig : 70

Table 28: *In-vitro* drug release of physical mixture in 1:2 in pH 4.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	33.8	32.4	31.2	32.4±1.2
10	39.7	38.6	41.3	39.8±1.4
20	48.5	44.9	46.6	46.6±1.8
30	59.6	58.2	62.1	59.9±1.9
45	67.4	64.0	66.5	65.9±1.7
60	74.6	71.8	75.9	74.1±1.4

In-vitro drug release of physical mixture 1:2 in pH 4.0

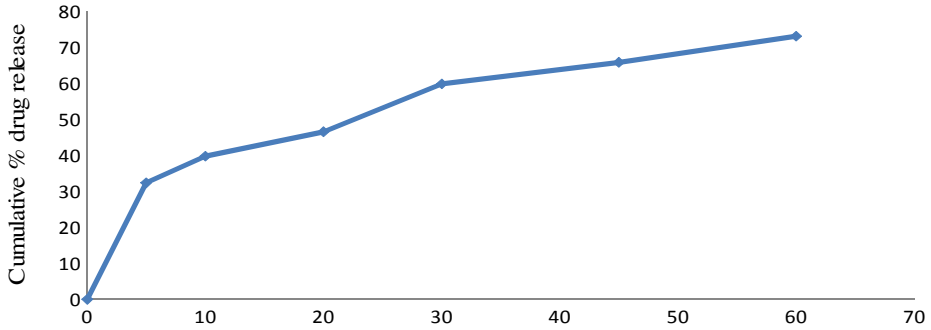


Fig : 71

Zero order release of physical mixture 1:2 in pH 4.0

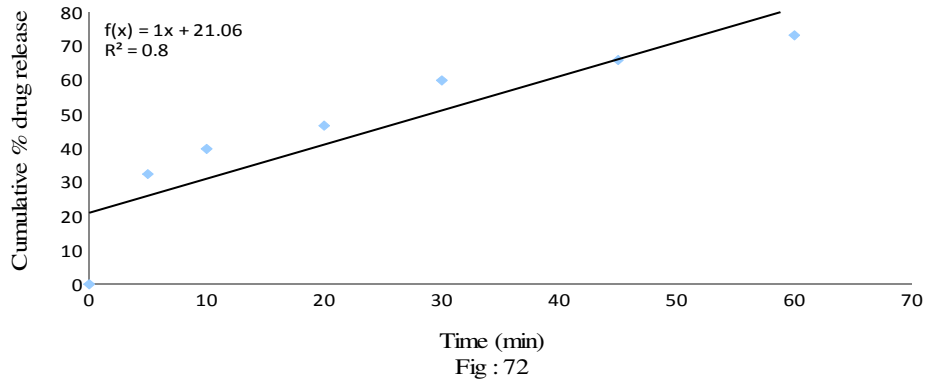


Fig : 72

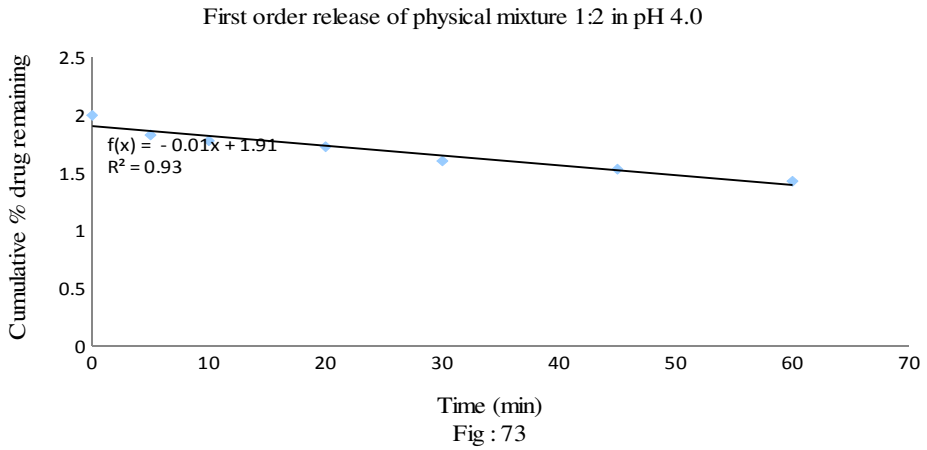


Fig : 73

Table 29: *In-vitro* drug release of co-precipitation1:2 in pH 4.0

Time (min)	Absorbance (nm)	Concentration (µg/ml)	Amount of drug release	Cumulative % drug release
5	40.4	38.6	41.5	40.16±1.4
10	1.451.7	54.3	52.9	52.96±1.3
20	66.3	67.9	65.8	66.6±1.0
30	69.6	71.4	72.1	71.0±1.2
45	74.5	78.5	76.3	76.4±2.0
60	79.9	83.5	84.3	82.5±2.3

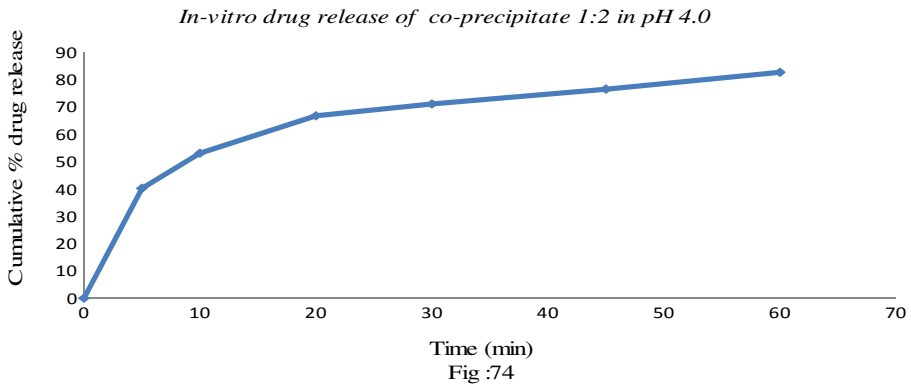


Fig :74

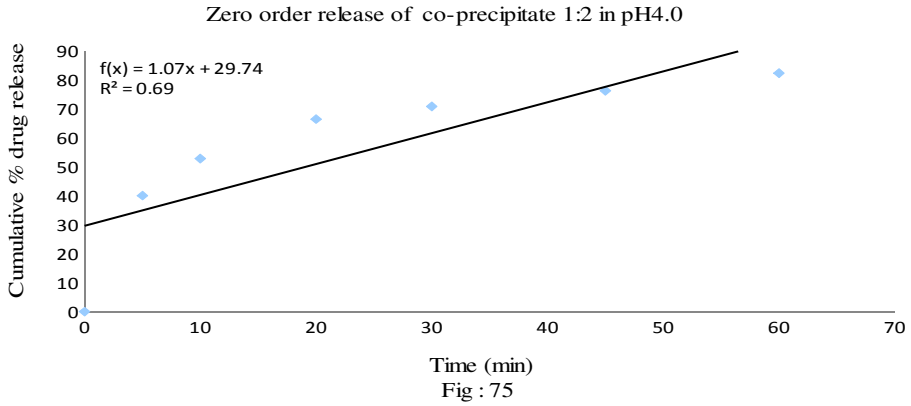


Fig : 75

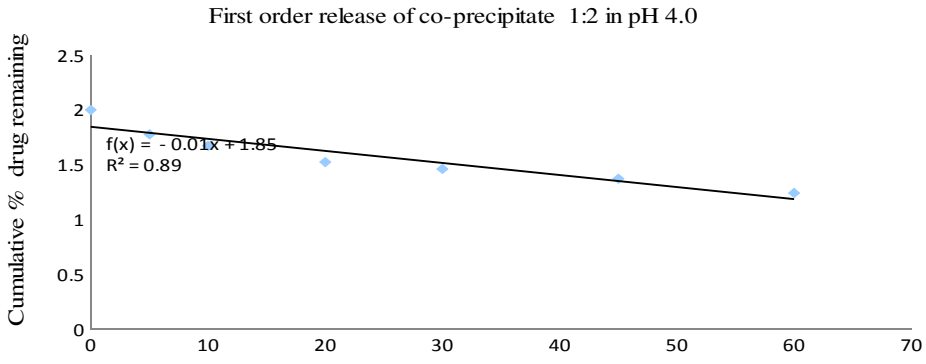


Fig : 76

Table: 30 *In-vitro* drug release of physical mixture 1:4 in pH 1.2

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	34.5	31.7	30.6	35.2±2.0
10	42.9	5.8	43.9	44.2±1.4
20	54.2	52.4	55.7	54.1±1.6
30	65.8	62.5	68.3	65.5±2.9
45	71.5	70.8	73.6	71.9±1.4
60	76.8	78.3	75.2	76.7±1.5

In-vitro drug release of physical mixture 1:4 in pH 4.0

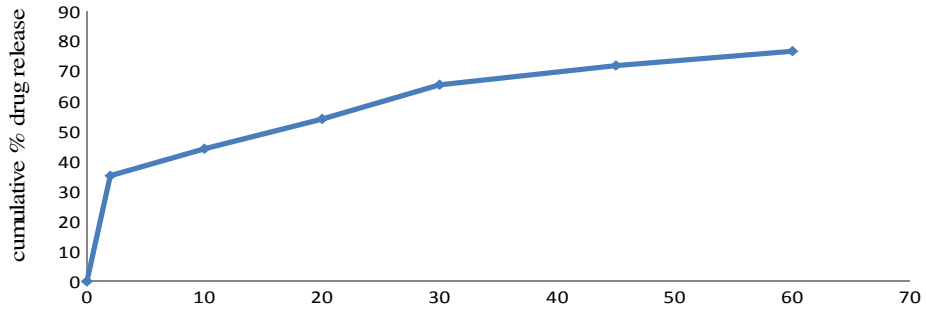


Fig :77

Zero order release of physical mixture 1:4 in pH 4.0

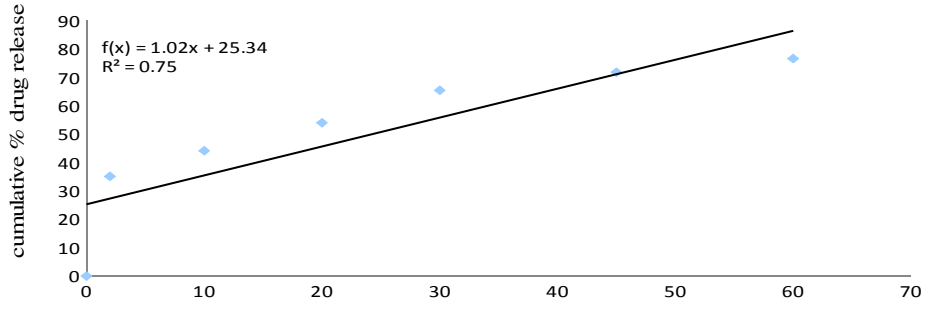


Fig : 78

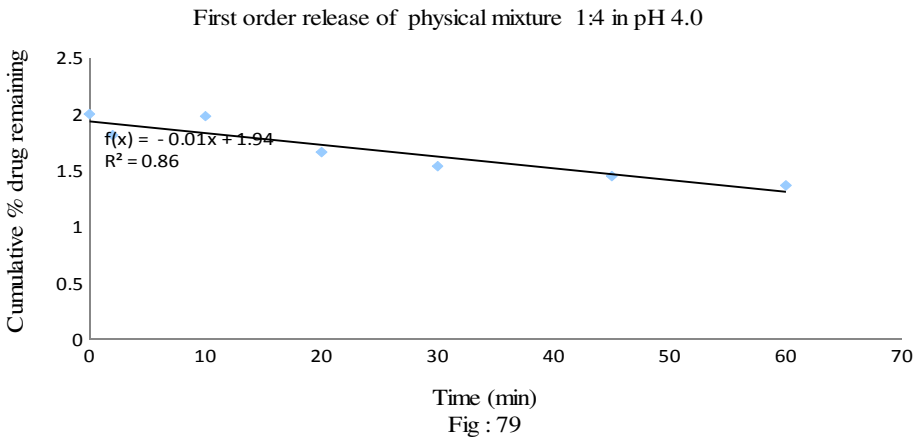


Fig : 79

Table 31: *In-vitro* drug release of Co-precipitation 1:4 in pH 4.0

Time (min)	Trial 1	Trial 2	Trial 3	Cumulative % drug release
5	33.6	32.6	35.4	33.8±1.4
10	43.8	44.6	42.6	43.6±1.0
20	51.4	53.8	54.4	53.2±1.5
30	66.4	62.4	65.8	64.8±2.1
45	71.8	73.8	70.5	72.0±1.6
60	85.9	86.2	88.3	86.8±1.308

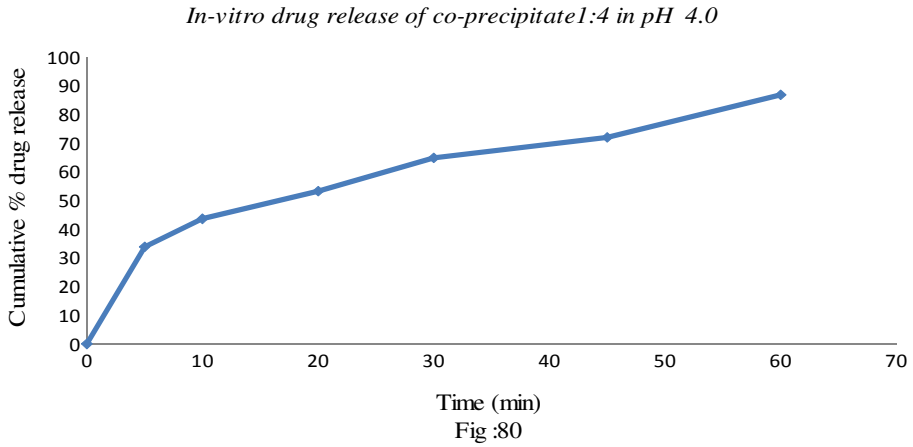


Fig :80

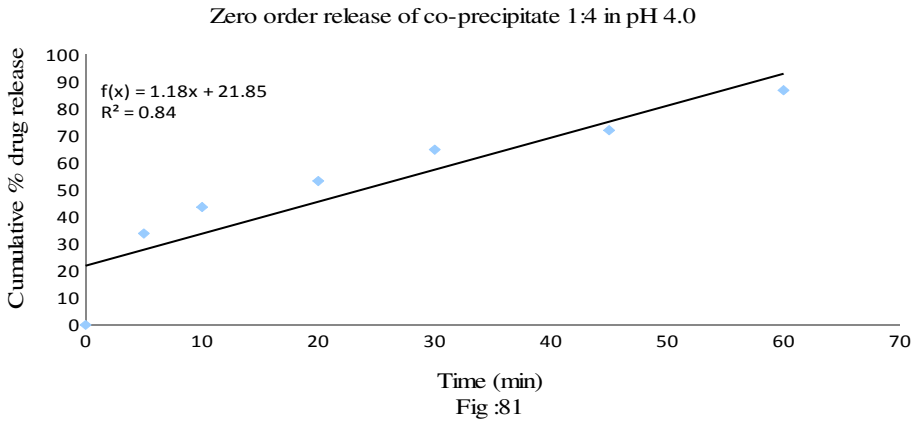


Fig :81

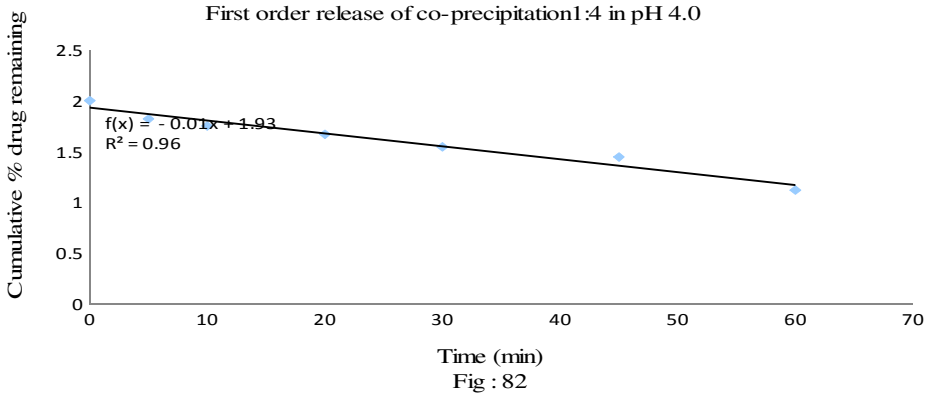


Fig : 82

Table 32: Comparative *In-vitro* dissolution of Itraconazole in different pH buffers

Time	Cumulative % drug release of Itraconazole			
	pH 1.2	**pH 2.0**	**pH 3.0**	**pH 4.0**
5	12.93±0.41	13.9±1.3	18.0±2.01	21.46±1.8
10	15.63±0.75	21.8±1.4	22.5±1.8	29.7±1.6
20	22.733±0.8	27.5±1.3	33.6±1.4	37.7±1.9

30	26.03±0.83	35.6±1.1	37.4±0.9	46.2±1.3
45	31.563±0.8	39.1±1.6	43.3±1.3	52.4±1.04
60	35.063±0.89	42.3±1.2	46.43±1.8	60.8±1.5

In- vitro drug release of ITC in different pH buffers

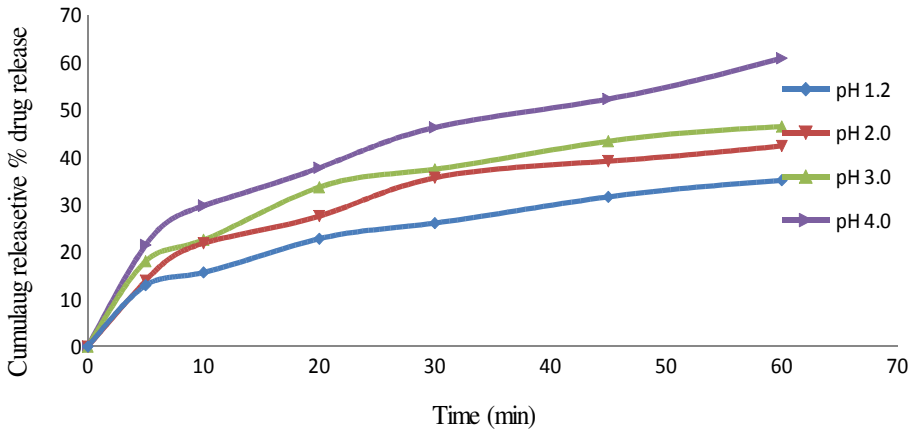


Fig.83

Table 33: Comparative *In-vitro* dissolution of ITC physical mixture in Different pH buffers

Time	Cumulative % drug release of physical mixture 1:2			
	pH 1.2	**pH 2.0**	**pH 3.0**	**pH 4.0**
5	26.4±0.81	23.2±1.6	17.0±1.8	32.4±1.2
10	29.48±0.87	28.9±1.1	25.5±1.1	39.8±1.4

20	31.6±0.86	36.7±1.8	34.4±o.5	46.6±1.8
30	36.33±0.94	45.2±1.4	46.6±1.2	59.9±1.9
45	47.36±0.66	56.4±1.9	54.8±1.3	65.9±1.7
60	57.3±0.61	59.6±2.8	62.9±1.0	74.1±1.4

In- vitro drug release of physical mixture 1:2 in different pH buffers

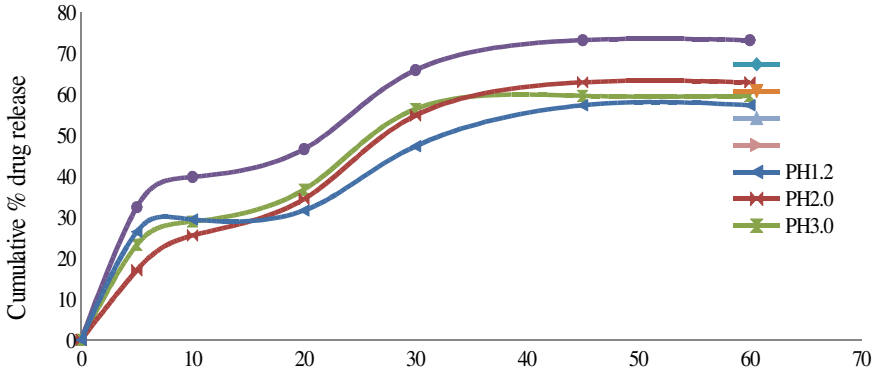


Fig : 84

Table 34: Comparative *In-vitro* dissolution of ITC Co- precipitate in different pH buffers

Time	Cumulative % drug release of co-precipitation 1:2			
	pH 1.2	**pH 2.0**	**pH 3.0**	**pH 4.0**
5	24.16±1.49	32.2±1.8	31.5 ±0.9	40.16±1.4
10	36.6±1.6	38.8±1.7	36.9 ±0.8	52.96±1.3
20	45.2±2.4	45.53±1.30	43.5 ±0.85	66.6±1.0
30	56.1±1.2	60.86±1.71	51.6 ±0.87	71.0±1.2
45	67.3±1.6	72.4±1.15	68.4 ±1.0	76.4±2.0
60	73.2±2.7	81.1±1.9	78.5 ±0.72	82.5±2.3

In- vitro drug release of co-precipitation 1:2 in different pH buffers

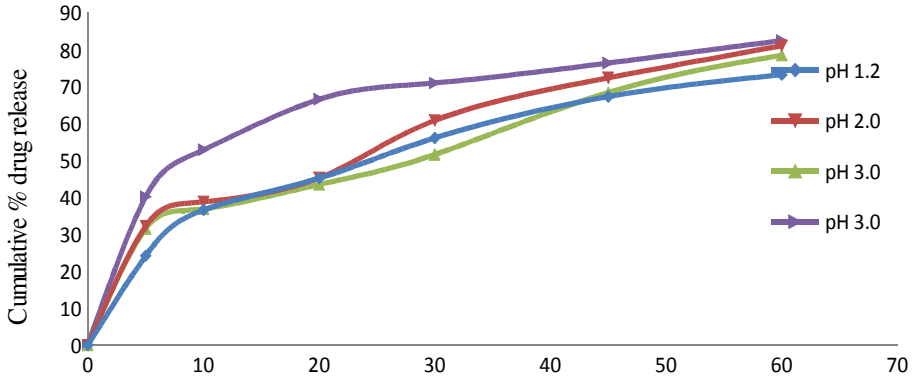


Fig :85

Table 35: Comparative *In-vitro* dissolution of ITC physical mixture 1:4 in different pH buffers

Time	Cumulative % drug release of physical mixture 1:4			
	pH 1.2	**pH 2.0**	**pH 3.0**	**pH 4.0**
5	24.8±1.4	21.8± 1.15	29.46±0.77	35.2±2.0
10	33.6±1.2	27.6±0.97	33.4±0.94	44.2±1.4
20	41.5±1.9	37.2±1.63	42,3±0.75	54.1±1.6
30	55.0±1.3	57.8±1.26	52.34±0.94	65.5±2.9
45	59.0±1.5	63.13±1.49	63.43±0.97	71.9±1.4
60	66.9±1.2	68.5±1.0	70.5±0.81	76.7±1.5

In- vitro drug release of physical mixture 1:4 in different pH buffers

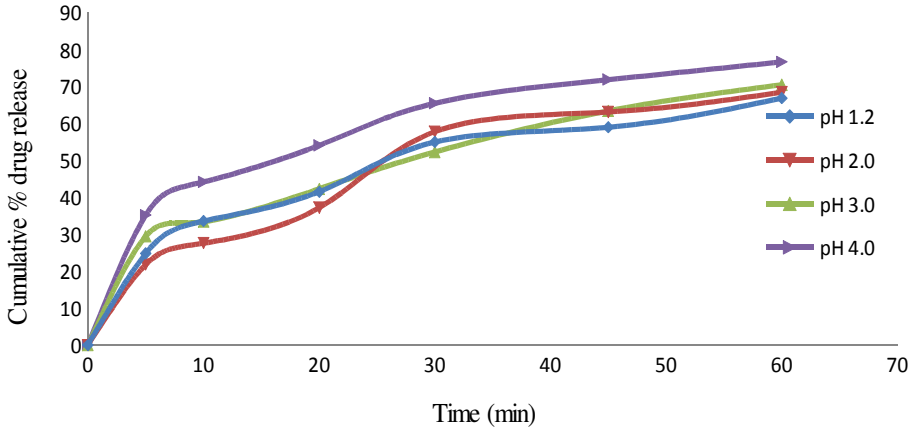


Fig . 86

Table 36: Comparative *In-vitro* dissolution of ITC co-precipitation 1:4 in different pH buffers

Time	Cumulative % drug release of co-precipitation 1: 4			
	pH 1.2	**pH 2.0**	**pH 3.0**	**pH4.0**
5	30.1±1.57	36.9±1.6	34.5±0.70	33.8±1.4
10	37.4±1.99	47.2±1.4	44.1±0.76	43.6±1.0
20	50.93±1.38	56.3±2.4	52.7±0.95	53.2±1.5
30	63.9±1.6	67.3±2.4	63.5±0.65	64.8±2.1
45	69.1±1.99	76.5±2.03	79.0±0.80	72.0±1.6
60	82.7±1.8	83.2±1.49	84.5±0.77	86.8±1.308

In- vitro drug release of co-precipitation 1:4 in different pH buffers

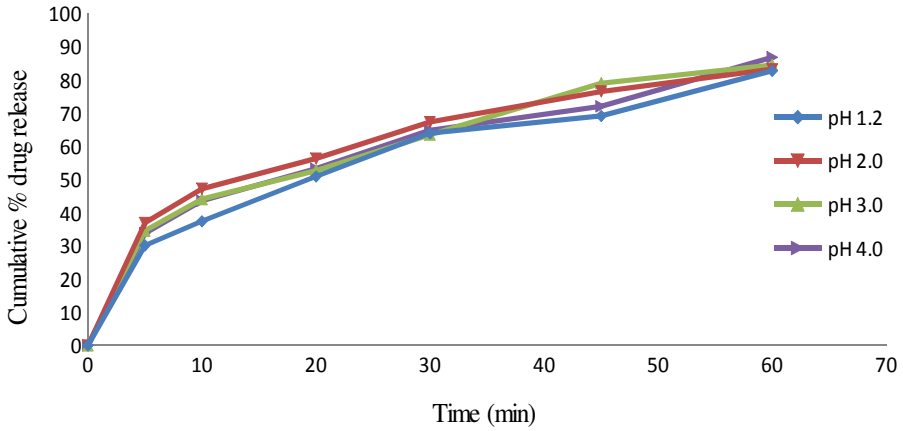


Fig : 87

Table 37: Comparative *In-vitro* dissolution study of different formulations in pH (1.2) buffer

Time	ITC	Cumulative % drug release in pH 1.2			
		PM 1:2	CO-PPT1:2	PM 1:4	CO-PPT 1:4
5	12.93±0.41	26.4±0.81	32.2±1.8	24.8±1.4	30.1±1.57
10	15.63±0.75	29.48±0.87	38.8±1.7	33.6±1.2	37.4±1.99
20	22.733±0.8	31.6±0.86	45.2±2.4	41.5±1.9	50.93±1.38
30	26.03±0.83	36.33±0.94	56.1±1.2	55.0±1.3	63.9±1.6
45	31.563±0.8	47.36±0.66	67.3±1.6	59.0±1.5	69.1±1.99

60	35.063±0.89	57.3±0.61	73.2±2.7	66.9±1.2	82.7±1.8

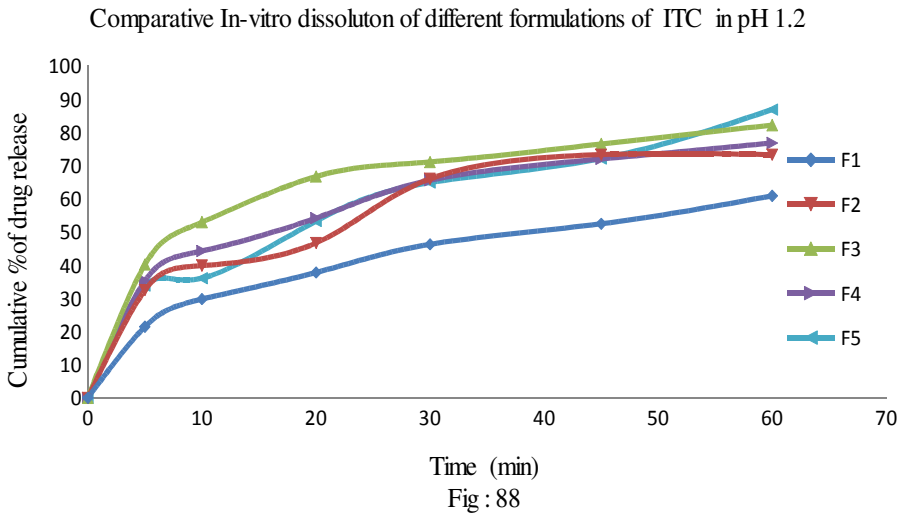


Fig : 88

Table 38: Comparative *In-vitro* dissolution of different formulations in pH (2.0) buffer

Time	ITC	Cumulative % drug release in pH 2.0			
		PM 1:2	CO-PPT1:2	PM 1:4	CO-PPT 1:4
5	13.9±1.3	23.2±1.6	24.16±1.49	21.8± 1.15	36.9±1.6
10	21.8±1.4	28.9±1.1	36.6±1.6	27.6±0.97	47.2±1.4
20	27.5±1.3	36.7±1.8	45.53±1.30	37.2±1.63	56.3±2.4
30	35.6±1.1	45.2±1.4	60.86±1.71	57.8±1.26	67.3±2.4

45	39.1±1.6	56.4±1.9	72.4±1.15	63.13±1.49	76.5±2.03
60	42.3±1.2	59.6±2.8	81.1±1.9	68.5±1.0	83.2±1.49

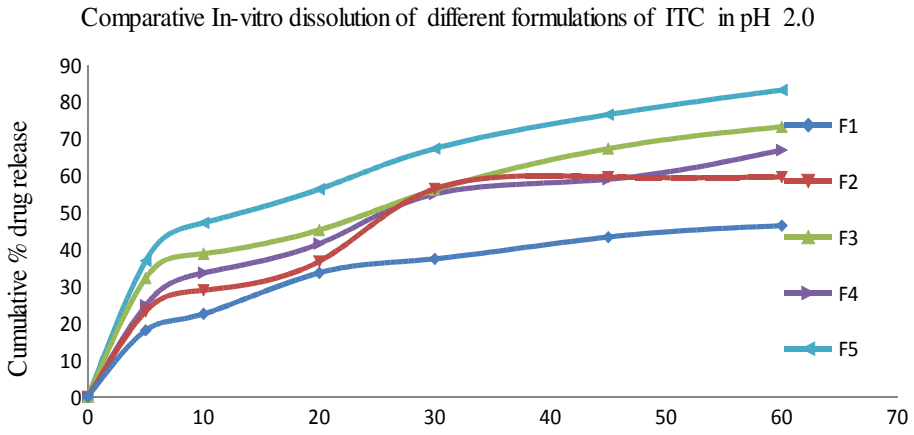


Fig : 89

Table 39: Comparative *In-vitro* dissolution of different formulations in pH (3.0) buffer

Time	ITC	Cumulative % drug release in pH 3.0			
		PM 1:2	CO-PPT1:2	PM 1:4	CO-PPT 1:4
5	18.0±2.01	17.0±1.8	31.5 ±0.9	29.46±0.77	34.5±0.70
10	22.5±1.8	25.5±1.1	36.9 ±0.8	33.4±0.94	44.1±0.76
20	33.6±1.4	34.4±o.5	43.5 ±0.85	42,3±0.75	52.7±0.95
30	37.4±0.9	46.6±1.2	51.6 ±0.87	52.34±0.94	63.5±0.65

45	43.3±1.3	54.8±1.3	68.4 ±1.0	63.43±0.97	79.0±0.80
60	46.43±1.8	62.9±1.0	78.5 ±0.72	70.5±0.81	84.5±0.77

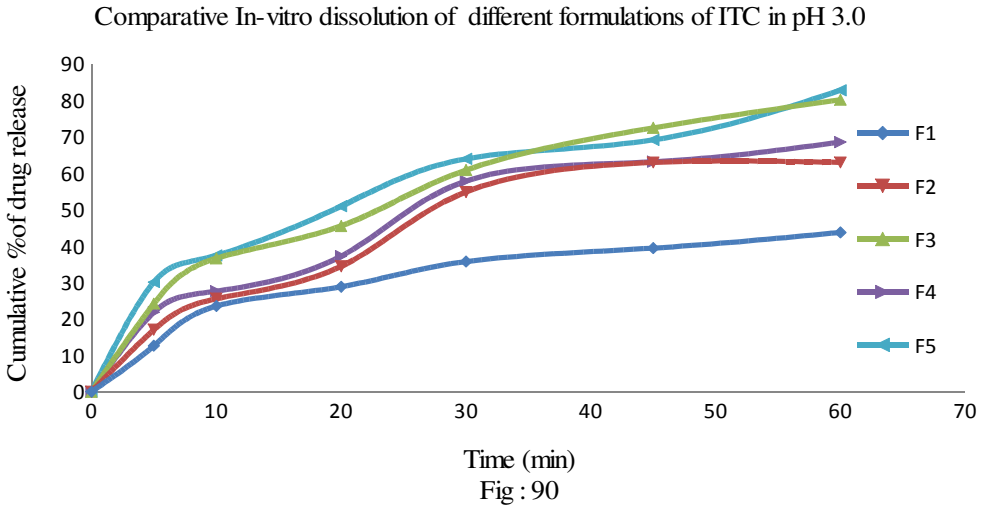


Fig : 90

Table 40: Comparative *In-vitro* dissolution of different formulations in pH (4.0) buffers

Time	ITC	Cumulative % drug release in pH 4.0			
		PM 1:2	CO-PPT1:2	PM 1:4	CO-PPT 1:4
5	21.46±1.8	32.4±1.2	40.16±1.4	35.2±2.0	33.8±1.4
10	29.7±1.6	39.8±1.4	52.96±1.3	44.2±1.4	43.6±1.0
20	37.7±1.9	46.6±1.8	66.6±1.0	54.1±1.6	53.2±1.5

30	46.2±1.3	59.9±1.9	71.0±1.2	65.5±2.9	64.8±2.1
45	52.4±1.04	65.9±1.7	76.4±2.0	71.9±1.4	72.0±1.6
60	60.8±1.5	74.1±1.4	82.5±2.3	76.7±1.5	86.8±1.308

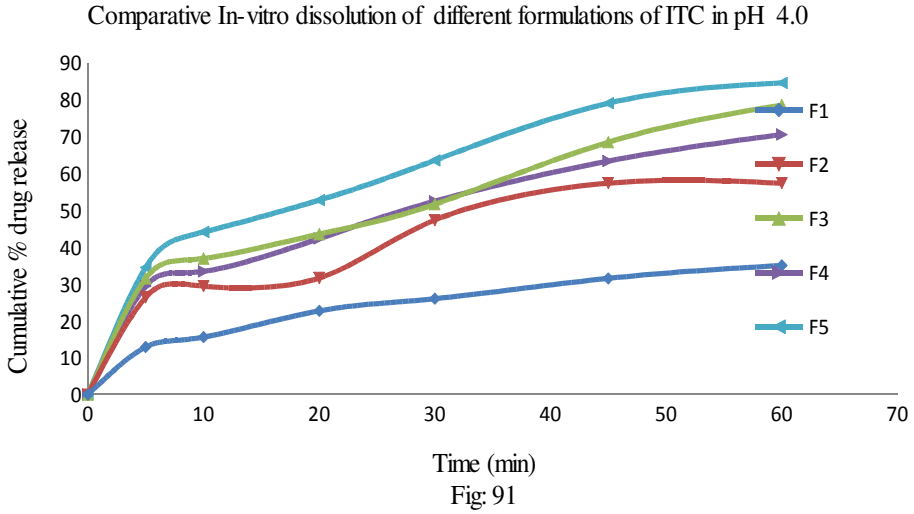
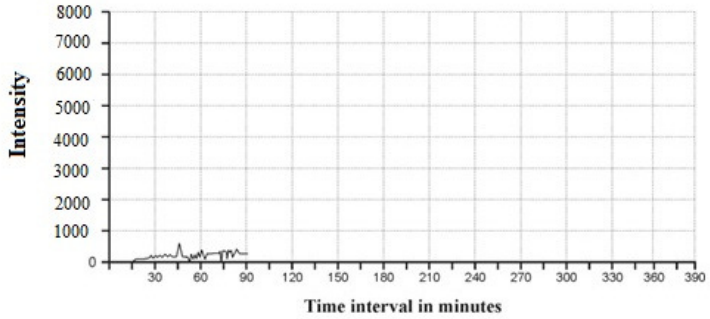


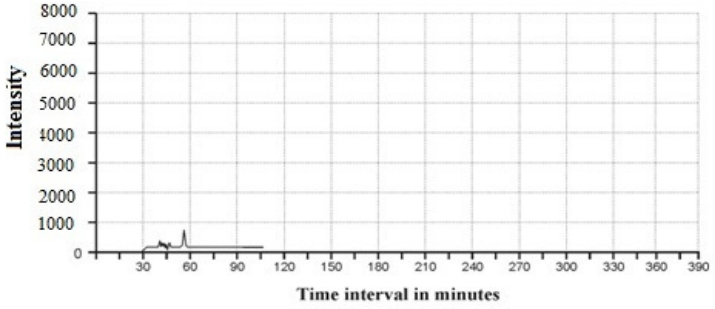
Fig: 91

8.5 *In-vivo* bioavailability of pure Itraconazole Physical mixture and co-precipitation 1:4 ratio.

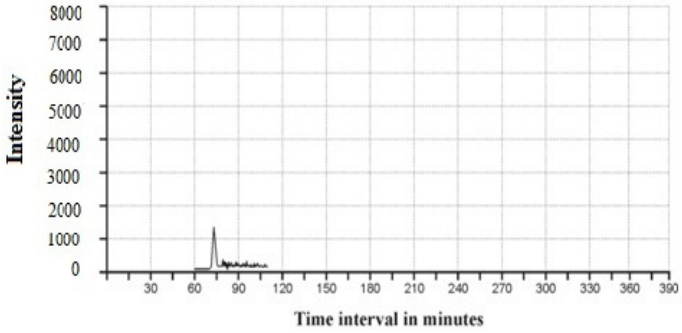
Pure Itraconazole Fig. 92



PLASMA SAMPLE (0 MIN)



PLASMA SAMPLE (30 MIN)



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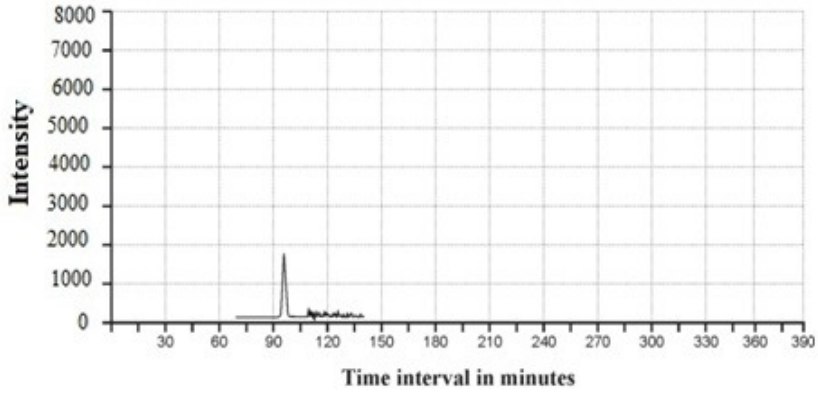

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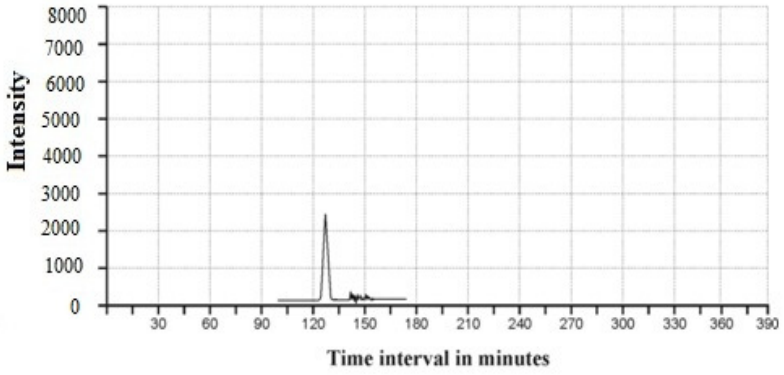
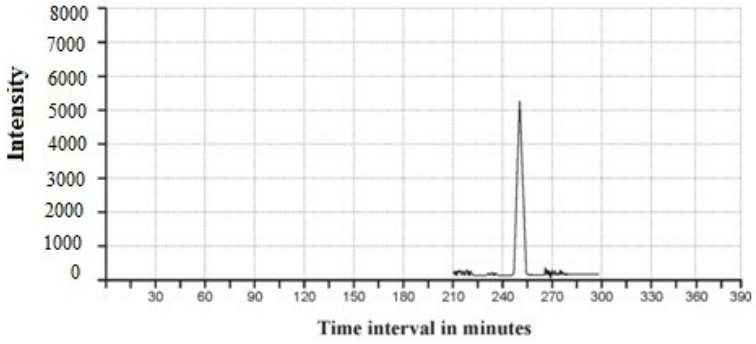
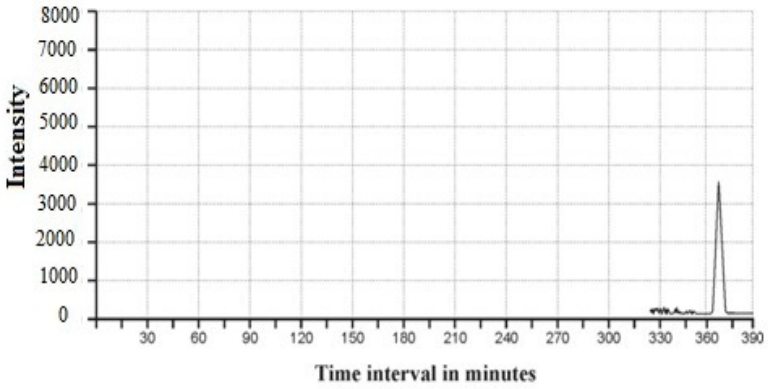


PLASMA SAMPLE (120 MIN)

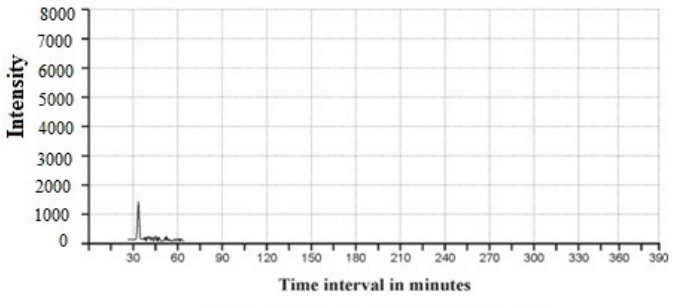


PLASMA SAMPLE (240 MIN)

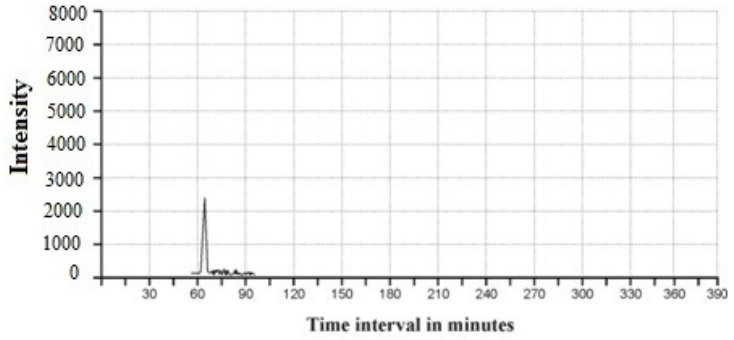


PLASMA SAMPLE (360 MIN)

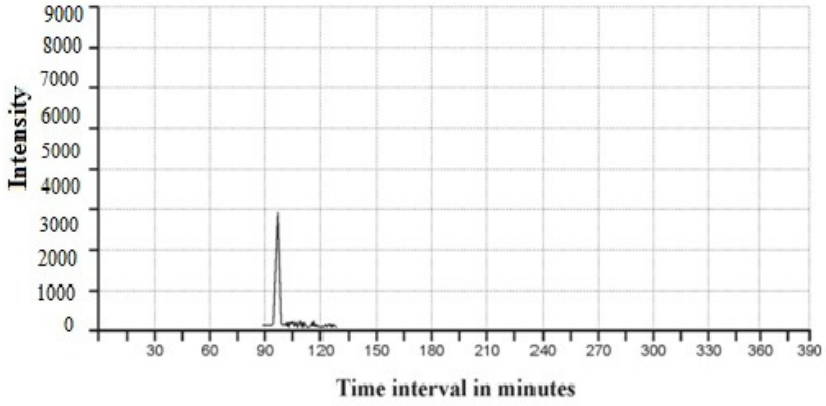
PHYSICAL MIXTUR 1:4



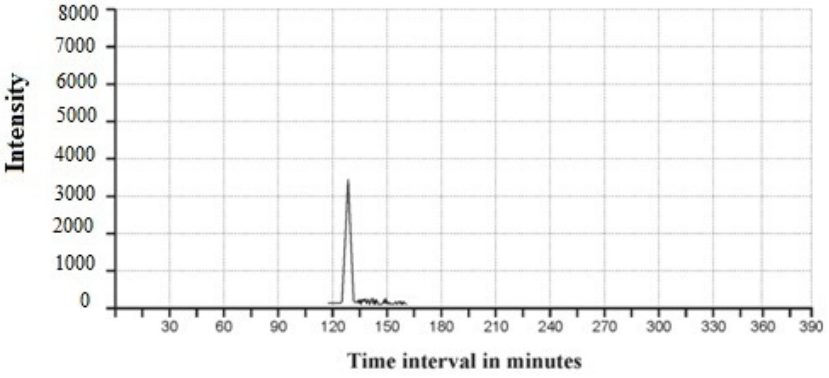
PHYSICAL MIXTURE (30 MIN)



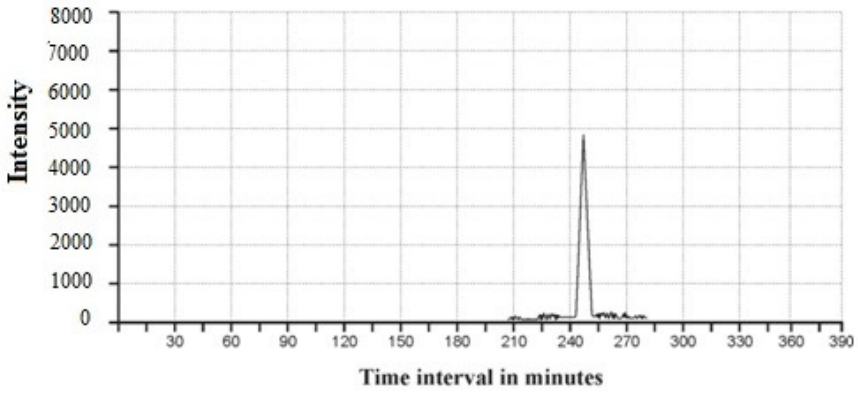
PHYSICAL MIXTURE (60 MIN)



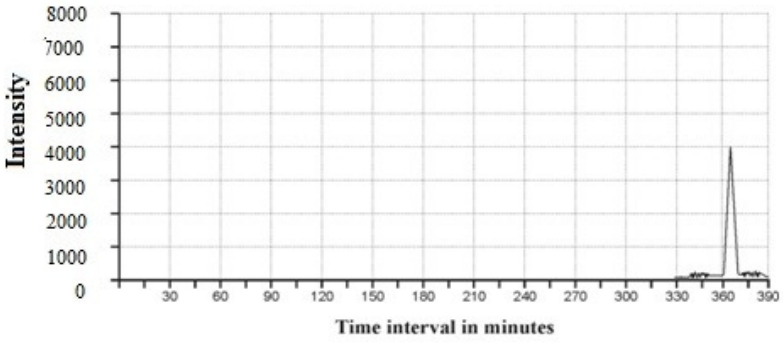
PHYSICAL MIXTURE (90 MIN)



PHYSICAL MIXTURE (120 MIN)

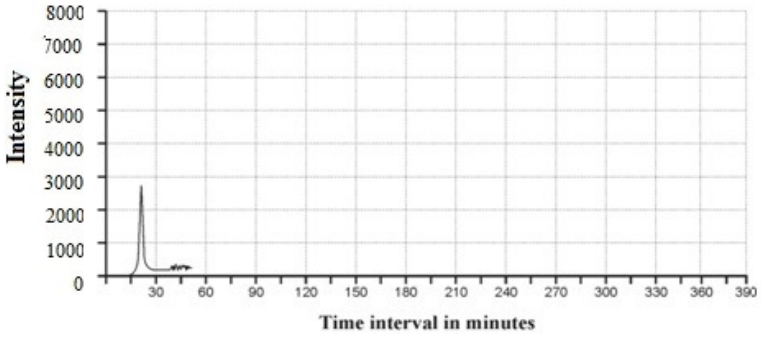


PHYSICAL MIXTURE (240 MIN)

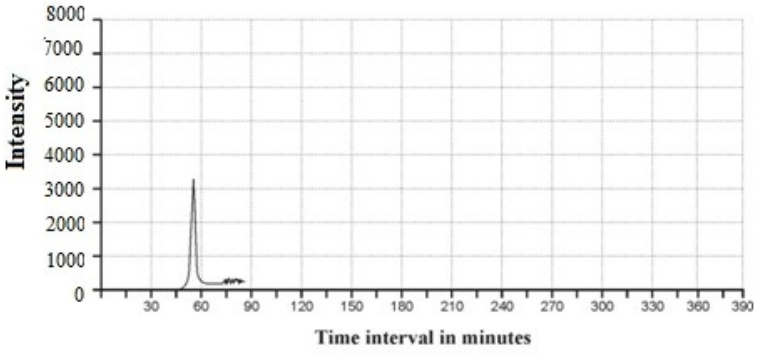


PHYSICAL MIXTURE (360 MIN)

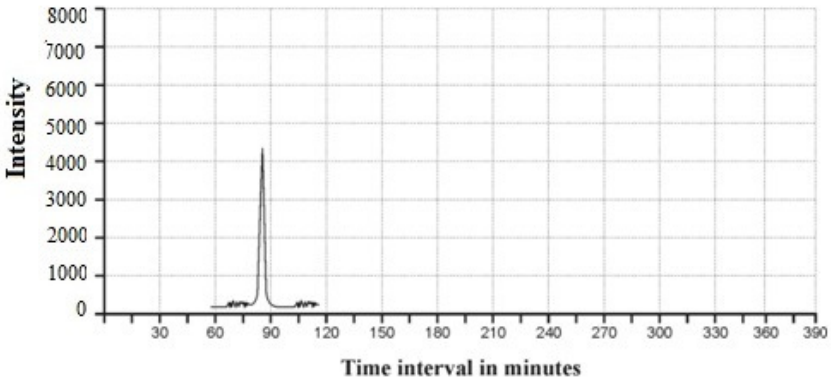
CO-PRECIPITATE 1:4 RATIO



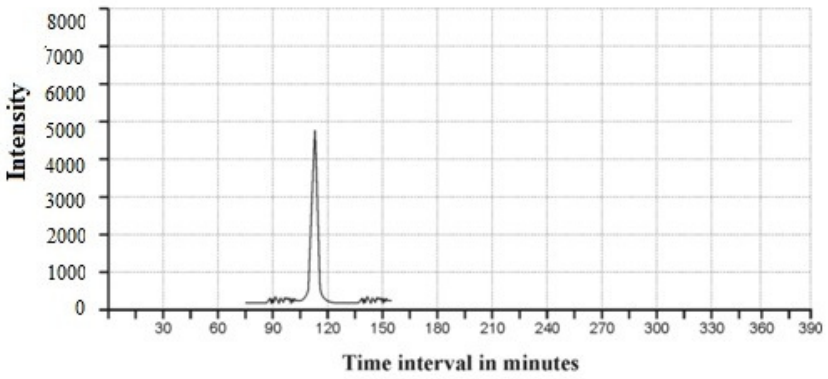
CO-PRECIPITATION(30 MIN)



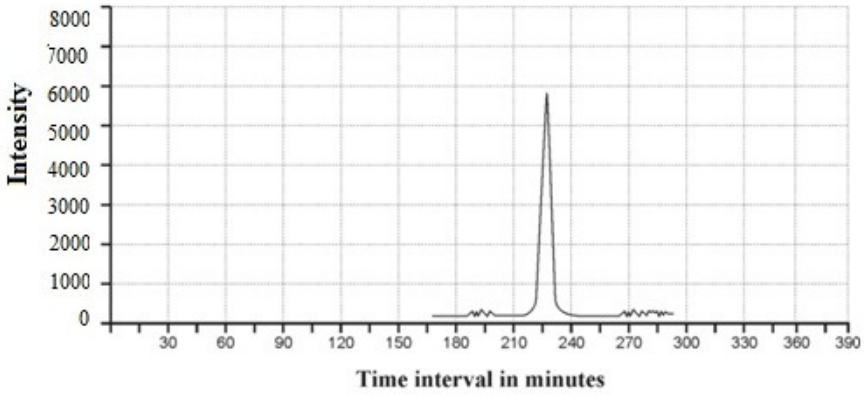
CO-PRECIPITATION(60 MIN)



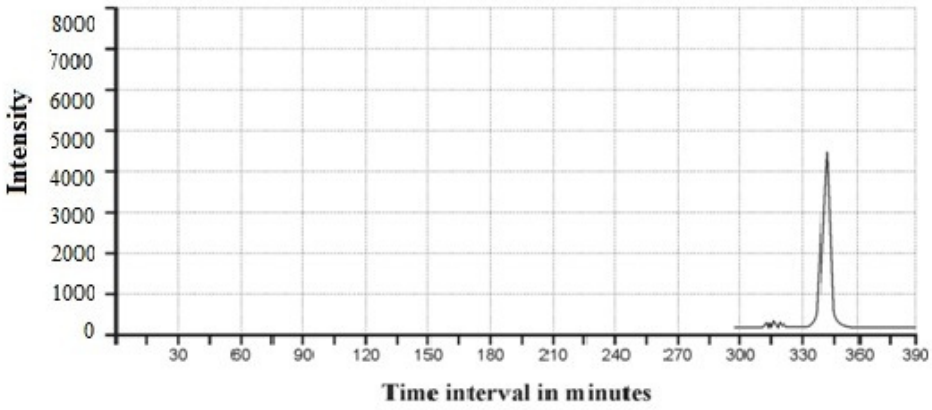
CO-PRECIPITATION (90 MIN)



CO-PRECIPITATION(120 MIN)



CO-PRECIPITATION(240 MIN)



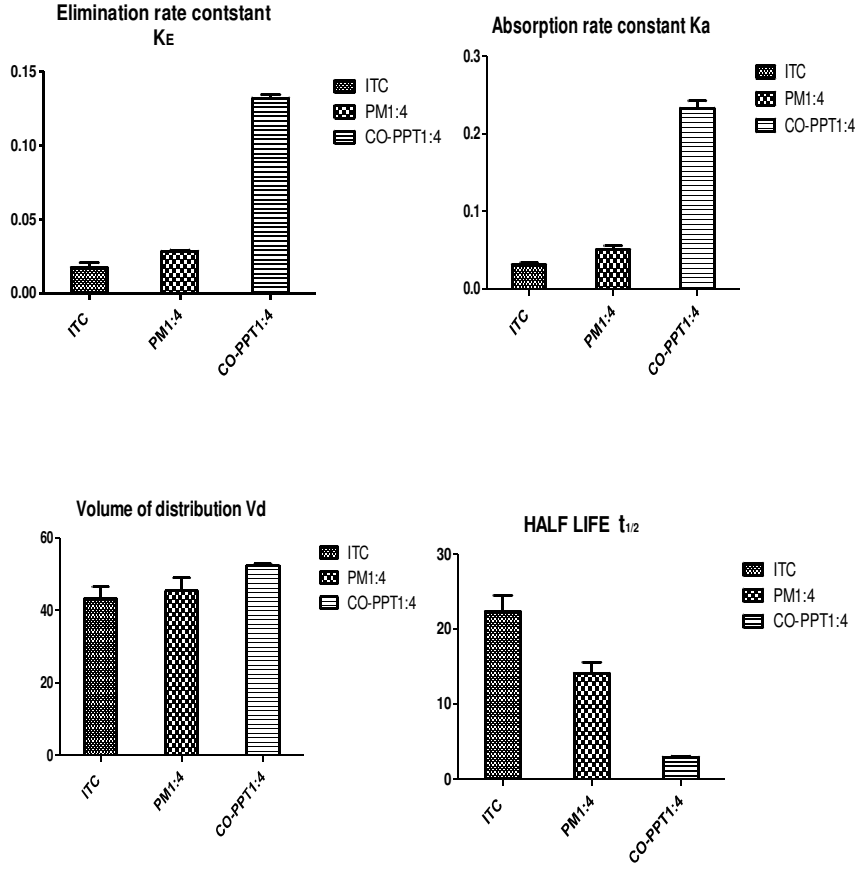
CO-PRECIPITATION(360 MIN)

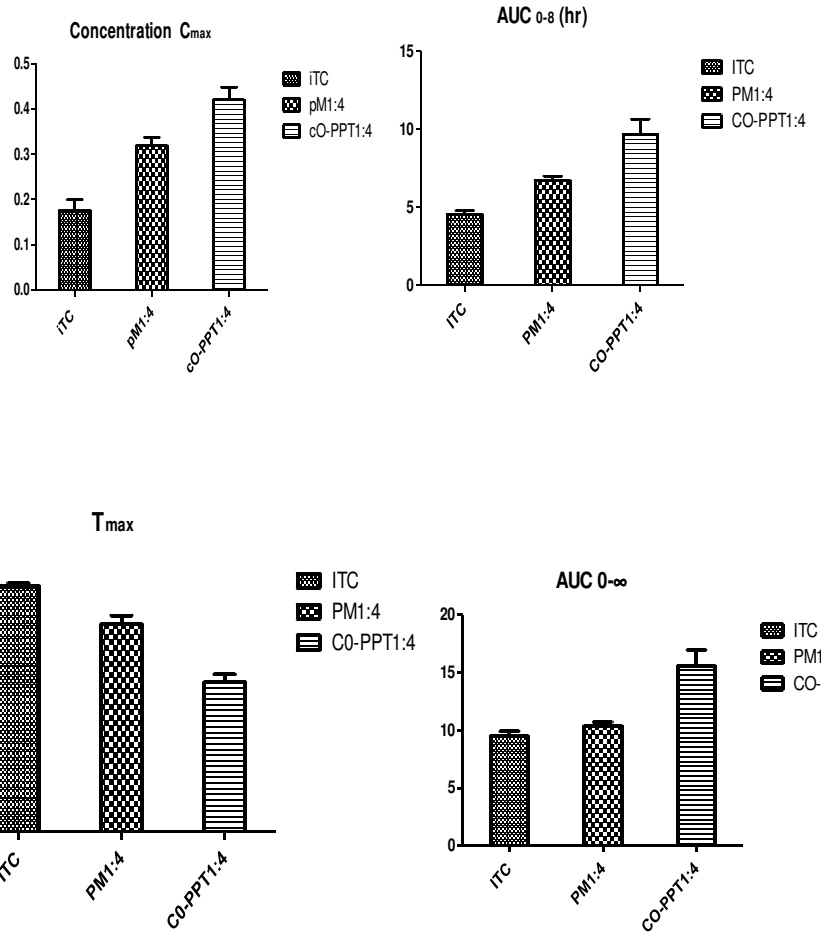
8.5 Comparative *In-vivo* pharmacokinetic study

Table 41: Comparative study of Pharmacokinetic parameters.

PARAMETERS	PURE±SEM	PHY MIX±SEM	CO-PPT±SEM	P- value
K_E	0.0176±0.00318	0.0286±0.00088	0.132±0.0023	***P<0.001
K_a	0.031±0.002728	0.0506±0.00504	0.233±0.0095	***P<0.01
$t_{1/2}$	22.4±2.095	14.16±1.462	2.96±0.1202	***P<0.001
V_d	39.2±3.3	42.19±3.57	45.4±0.4583	N.S
C_{max}	0.176±0.0233	0.32±0.01732	0.42±0.02906	***P<0.001
T_{max}	2.746±0.02728	2.32±0.0933	1.67±0.0895	***P<0.01
AUC_{0-8}	4.57±0.2083	6.72±0.2800	9.7±0.9528	***P<0.001
$AUC_{0-\infty}$	9.53±0.3756	10.4±0.3215	15.6±1.365	***P<0.001

Fig .93 GRAPHICAL REPRESENTATIONS OF PHARMACOKINETIC PARAMETERS




Concentration Cmax
0.5
0.4
0.3
0.2
0.1
0.0
iTC
pM1:4
cO-PPT1:4
iTC
pM1:4
cO-PPT1:4
AUC 0-8 (hr)
15
10
5
0
ITC
PM1:4
CO-PPT1:4
ITC
PM1:4
CO-PPT1:4
Tmax
3
2
1
0
ITC
PM1:4
C0-PPT1:4
ITC
PM1:4
C0-PPT1:4
AUC 0-∞
20
15
10
5
0
ITC
PM1:4
CO-PPT1:4
ITC
PM1:4
CO-PPT1:4

CHAPTER - IX

DISCUSSION

9. RESULTS AND DISCUSSION

FTIR spectra of all formulations

The FTIR spectra for pure Itraconazole, pure β- cyclodextrin, physical mixture, and co-precipitate of Itraconazole and β- cyclodextrin (1:2 and 1:4) are shown in table 6 figure 3-8. The characteristic FTIR spectra of Itraconazole and β- cyclodextrin were observed. In physical mixture's indicating no complex formation or interaction between Itraconazole and β- cyclodextrin, physical mixture, however in co-precipitate the spectra of dimethylalkane and α, β, 5- member ring were absent this shows complex formation between Itraconazole and β- cyclo dextrin .

The results of the FTIR spectra shows that an inclusion complex was formed between Itraconazole and β- cyclo dextrin by co-precipitation method and physical mixture of Itraconazole and β- cyclo dextrin did not bring about interactions between the drug and β- cyclo dextrin. The results of FTIR spectra shows that an inclusion complex was formed between Itraconazole and β- cyclodextrin by co-precipitation method and physical mixing of Itraconazole and β- cyclodextrin did not bring about interaction between the drug and β- cyclodextrin.

Differential scanning calorimetry (DSC)

DSC curves for pure Itraconazole pure β-cyclodextrin, physical mixture and co-precipitate are (1:2 and 1:4) ratios of Itraconazole and β-cyclodextrin are shown a figure 9-14 Pure Itraconazole shows a sharp melting endoderm at 168.9°c. Pure β-cyclodextrin showed two peaks a broad endothermal effect ranging between 50- 100°c corresponding to dehydration of β-cyclodextrin and second at 245°c characteristic of pure β-cyclodextrin. DSC curves of the physical mixture for both drug: β-cyclodextrin ratios (1:2 and1:4) consists of thermal profiles of drug and cyclodextrin with no significant changes in peaks showing no drug cyclodextrin interactions. DSC curves for the co-precipitate showed slight broadening of endothermal peak for β-cyclodextrin at 245°c and more broadening of the endothermal peak for β-cyclodextrin between 50 - 150°c indicative of drug – cyclodextrin interaction. The endothermal peak corresponding to pure β-cyclodextrin was more broader in the co-precipitate with 1:4 (dug: cyclodextrin) compared to that for 1:2 (dug: cyclodextrin) ratio. These findings suggest higher inclusion yield for the co-precipitate having 1:4 (drug: β-cyclodextrin).

Phase solubility

The effect of β-cyclodextrin in different concentrations on the solubility of the Itraconazole was examined and the results are shown in the table and figures. 7 and 15-18 respectively. The solubility of Itraconazole decreased as pH increases from 1.2- 4.0. Itraconazole being weakly basic in character becomes more un-ionised in higher pHs. Which is supportive of earlier finding that absorption is enhanced by concurrent administration of food possibly due to increase in gastric pH by the effect of food and thus establishing the unionized form are more absorbable. Solubility of Itraconazole as increased propionately with increase in the con of β-cyclodextrin in pH 1.2 medium and this increase was reduced as pH increased.

The increase in solubility by the effect of cyclodextrin may be that structure of cyclodextrin is analogous to a truncated cone with a hydrophobic interior and hydrophilic exterior, This allows the mode of encapsulatution of hydrophobic portions of gust molecules thus shielding them from the polar forces of aqueous solutions. The increased solubility of Itraconazole by the effect of β-cyclodextrins may also be through non-inclusion complex formation, on finding is in consistent with earlier report that cyclodextrin complexes can self associate to form aggregate through non- inclusion complex by micelle like structure, which also effectively solubilize poorly water soluble drugs.[18]

Previously it has been reported from the phase solubility study 2-oH- propyl β-cyclodextrin improves solubility of Itraconazole in aqueous solution.20 The results of the phase solubility study clearly suggest that solubility of Itraconazole though improved by β- cyclodextrins, it decreases in higher gastric pH condition which is normally seen after taking food.

In-vitro dissolution

Dissolution profile of pure Itraconazole, physical mixture and co-precipitate, Itraconazole β-cyclodextrin, (1:2 and1:4) is shown i n the tables 8- and figure 15-86. The % drug release increased over time and reached above 35% in pH1.2 medium and as the pH of the medium increased the % drug release also increased thus showing the effect of pH on the dissolution of Itraconazole as it has been reported earlier[6] absorption of Itraconazole is enhanced by the concurrent administration of food and the gastric pH increase in presence of food and so my be the effect of enhanced dissolution of Itraconazole in higher acidic pHs as observed in the present study. Dissolution amounts were more from physical mixture as compare to pure Itraconazole and the dissolution amounts of physical mixture of 1:4 (drug: β-CD) were more than that of physical mixture of 1:2 (drug: β-CD) ratios. These findings are in consisting with our findings in phase solubility study where the solubility of Itraconazole increased as concentration of β-CD increase so the higher dissolution amount with higher con of β-CD is due to enhanced solubility of Itraconazole by the effect of β-CD. The solid state of drug was not effected in the physical mixture as observed in the thermogram and as such the influence of physical state of drug on the dissolution of Itraconazole is less likely. Dissolution amount of co-precipitate was higher than that of pure drug as well as physical mixtures. Co-precipitate1:4 (drug: β-CD) ratio showed higher dissolution amounts as compared to that of co-precipitate 1:2 (drug: β-CD) ratio as evident from DSC. The endothermic peak of β-CD was more broader in co-precipitate 1:4(drug : β-CD) than in co-precipitate 1:2 (drug: β-CD) as the evident from the DSC thermo gram, suggesting more inclusion complex formation with higher concentrations of β-CD's and therefore higher dissolution amounts with higher con of β-CD.

Comparison of dissolution profile of physical mixture and co-precipitate at varying acidic pH's shows maximum dissolution amounts from co-precipitate 1:4 (drug: β-CD) as compared to other formulation and dissolution amounts of co-precipitate of 1:4 (drug :β-CD) increased as the pH increased in the same pattern of increased dissolution amounts of physical mixture and co-precipitate1:2 (drug: β-CD) ratio was observed. Physical mixture 1:4 (drug: β-CD) showed higher dissolution amount compare to

physical mixture 1:2 (drug: β-CD). Similarly co-precipitate1:4 (drug: β-CD) showed higher dissolution amount compared to co-precipitate 1:2 (drug: β-CD). The highest dissolution of Itraconazole from co-precipitate 1:4 (drug: β-CD) sis due to more inclusion complex formation observed in the DSC thermo gram. β -cyclodextrin in increased concentrations increases the solubility as well as dissolution of Itraconazole from both physical mixture and co-precipitation. The increased dissolution of Itraconazole either from the physical mixture or from the co-precipitate in higher acidic ph suggest that absorption of Itraconazole is enhanced by β-CD, and this absorption is further influenced by improved dissolution of Itraconazole at higher acidic pH from physical mixture as well as co-precipitate.

The results of dissolution study proposes that β-CD can improve the dissolution, absorption and bioavailability of Itraconazole and these factors are influenced by pH of acidic environment of the stomach.

In-vivo pharmacokinetics

Based on dissolution profile of the formulations the physical mixture 1:4 (drug: β-CD) and co-precipitate 1:4 (drug: β-CD) were selected as the best formulation and the in-vivo pharmacokinetics of these formulations were studied. Plasma con of Itraconazole, physical mixture and co-precipitate after oral admin are shown in table 40 and figure 91-98. Ke, Ka, Cmax, AUC_{0-8}, and AUC 0-∞, showed higher values from physical mixture and co-precipitate as compared to that of pure Itraconazole and these values were higher with co-precipitation as compared to physical mixture that showing the formulation of inclusion complex in the co-precipitate resulting in improved dissolution and pharmacokinetics of co-precipitate. The $t_{1/2}$ and Tmax of co-precipitate were significantly lower and that of physical mixture as well as pure Itraconazole, thus further supporting the enhanced absorption of Itraconazole from the co-precipitate through inclusion complex formation.

CHAPTER - X

SUMMARY & CONCLUSION

10. SUMMARY AND CONCLUSION

- Co-precipitation of 1:4 (drug: β-CD) showed higher dissolution as compared to co-precipitation 1:2 (drug: β-CD) physical mixture (1:2,1:4) (drug: β-CD) and pure Itraconazole
- Dissolution amounts of co-precipitate increased as the pH of acidic environment increased.
- Solubility of Itraconazole was improved with higher concentrations of β-CD.
- Inclusion complex formation was detected in co-precipite's as observed in the DSC thermograms. Physical mixture did not show interaction between Itraconazole and β-CD as supported by the results of FTIR spectra and DSC analysis.
- The enhanced dissolution of Itraconazole from co-precipitate of 1:4 (drug: β-CD) was reflected in improved pharmacokinetics of co-precipitate.
- The results of the study proposes that the dissolution and bio-availability of Itraconazole can be improved by using β- cyclodextrin, how ever bioavailability of Itraconazole can be influenced by the pH conditions of the gastric environment as evident from dissolution study.
- Further studies are warranted to examine the effect of food on the bio availability of Itraconazole-β-cyclodextrin complex.

CHAPTER - XI

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